

Causes of deafness in East London Bangladeshi children

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Submitted for the degree of
MD

September 2007

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Declaration

The work presented in this thesis “Causes of deafness in East London Bangladeshi population” is my own work conducted under the supervision of Dr M Bitner-Glindzicz and Mr D Albert.

Yogesh Bajaj

Abstract

The aim of this study was to examine the causes of sensorineural hearing loss in the Bangladeshi population resident in East London. Almost all of this population originates from Sylhet, a province in Bangladesh. The study was conducted at a community based audiology clinic and tertiary level genetics department. One hundred and fifteen families (134 patients) were ascertained; 11 families declined to participate and 4 families could not be contacted. All children of Bangladeshi ethnic origin with bilateral sensorineural hearing loss more than 40dB in the better hearing ear were included in this study. Information on all these patients was collected from their case notes. For the 67 patients in whom the cause of deafness was not clear from the records or unknown (or non-syndromic deafness), families were seen in the research clinic.

The prevalence of deafness >40db in Bangladeshi children under 16 years of age in East London was calculated to be approximately 3.86 per 1000 (95%CI: 3.24, 4.47). This is nearly 2.3 times the national average. Parents were consanguineous in 35 out of 105 families (33.3%) in which this information was available. On calculating the prevalence of deafness in the Bangladeshi children belonging to non-consanguineous families, the prevalence falls to 2.72 per 1000 (95%CI: 2.10, 3.34). Genetic causes appear to be the most common cause of deafness in Bangladeshi population in 59.6% patients. Environmental causes were responsible for hearing loss in 18.5% patients and in 21.8% cases the cause of deafness was undetermined. Of the deafness due to genetic causes, 57.7% were non-syndromic, 25.3% syndromic and 16.9% were chromosomal.

The single most common cause of sensorineural hearing loss in the Bangladeshi population in this study was due to mutations in the *GJB2* gene (Connexin 26) in 14 of

these families. The mutations in *GJB2* in this population were W24X, IVS1+1G→A, M1V, V95M and W77X. W24X was the most common mutation seen in 40% (8/20) patients. Genetic causes are the common cause of deafness in subjects of Bangladeshi origin and 29.8% children with non-syndromic deafness were positive for mutations in *GJB2*.

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Acknowledgements

Firstly I would like to thank my supervisors Dr M Bitner-Glindzicz, Reader Clinical & Molecular Genetics and Mr D Albert, Consultant Otolaryngologist for their constant support, guidance and encouragement without which I would have never been able to complete this research project and the thesis.

I would also like to thank Dr T Sirimanna, Consultant Audiological Physician for his advice and help throughout this project. I am also grateful to Ms M Garside, Coordinator & Teacher of Deaf for providing me the list of Bangladeshi patients on her records.

I am deeply indebted to Ms Parveen Quader, Bengali Liaison Worker, as without her help in translating during recruitment and clinic consultation this study would not have been possible. My sincere thanks are due to Ms S Sattar, Bengali Link Worker, for her help in translating and audio recording of information leaflet.

I am also very grateful to all the team members of Regional Clinical Molecular Genetics Lab specially Dr L Jenkins, Clinical Scientist, for analysis of the research samples. I would also like to thank all the Audiological Scientists at Donald Winnicott Centre specially Mr P Premachandra for their cooperation in running the research clinic.

I am grateful to Dr R Chorbachi & Dr K Rajput, Consultant Audiological Physicians for allowing me to recruit their patients for this research.

I would like to profusely thank all my patients and their parents without whose cooperation and consent this research could not have been completed.

Finally I would like to say a big thanks to my mother Mrs U Bajaj, my parents in law Mr B Moitra & Mrs S Moitra, my wife Monika and my son Gaurav and daughter Keya for their love and cooperation.

Yogesh Bajaj

Definitions

The terminology used in this thesis (Fortnum *et al.* 2001):

Congenital impairment: Hearing loss that is recognized at birth or believed to have been present since birth.

Late onset impairment: Hearing loss that first manifests itself after birth and cannot be attributed to an identifiable exogenous cause.

Progressive impairment: Deterioration in hearing greater than or equal to 15dB in the pure tone average within a ten year period.

Acquired impairment: Hearing loss that first manifests after birth and can be attributed to an identifiable exogenous cause.

Confirmation of

hearing impairment: This is the outcome of the process of establishing that a child has hearing loss.

Ascertainment: The identification of a child from one or more notifications by the research team.

Total ascertainment: This is the process of attempting to ascertain all cases in the population.

Prevalence of hearing loss: The number of children per thousand live births in an annual birth cohort with confirmed permanent bilateral hearing loss.

The definitions used for different types of hearing loss are (Davis & Mencher 2002)

- Sensorineural:** Hearing loss due to disease or deformity of the inner ear or cochlear nerve with an air bone gap of less than 15dB averaged over 0.5,1 and 2 KHz.
- Conductive:** Hearing loss due to disease or deformity of the external or middle ear with an air bone gap of more than 15dB and normal bone conduction (<20dB) averaged over 0.5,1 and 2 KHz.
- Mixed:** Hearing loss due to combined involvement of middle or external ear and inner ear or cochlear nerve. The audiogram shows bone conduction thresholds greater than 20dB and air bone gap more than 15dB averaged over 0.5,1 and 2 KHz.
- Sensory:** This is a subdivision of sensorineural hearing loss due to disease or deformity in the cochlea.
- Neural:** This is a subdivision of sensorineural hearing loss due to disease or deformity in the cochlear nerve.
- Central:** This is a sensorineural hearing loss due to disease or deformity in the central nervous system cephalic to the cochlear nerve.
- Average hearing level:** This is an average of the level of hearing thresholds measured in the better hearing ear at 0.5,1,2 and 4 KHz.
- Mild hearing loss:** An average hearing level of between 20-39dB (Stephens 2001).
- Moderate hearing loss:** An average hearing level of between 40-69dB (Stephens 2001).
- Severe:** An average hearing level of between 70-94dB (Stephens 2001).
- Profound:** An average hearing level of more than 95dB (Stephens 2001).

Asymmetrical hearing loss: This refers to a greater than 10dB difference between the ears in at least two frequencies with the average hearing threshold more than 20dB in the better hearing ear.

Low frequency ascending: More than or equal to 15dB between poorer low frequency thresholds and high frequencies.

Mid frequency loss: More than or equal to 15 dB between poorest mid frequency thresholds and low and high frequencies.

Flat loss: Less than 15dB difference between mean of 250/500 Hz, mean of 1 & 2 kHz and the mean of 4 and 8 kHz thresholds.

Gently sloping: 15-29dB difference between 500-1000Hz mean and 4000-8000Hz mean.

Steeply sloping: More than or equal to 30dB difference between 500-1000Hz mean and 4000-8000Hz mean.

Progressive hearing loss: More than or equal to 15dB deterioration over a 10 year period in the average of 0.5, 1, 2 and 4 KHz frequencies.

Loci for genes are defined as:

DFNA	Autosomal recessive
DFNB	Autosomal dominant
DFN	X linked

1 Introduction

Effect of hearing on language & social development

Hearing impairment has a big impact on the development of language and communication. It can lead to poor literacy skills (Davis & Mencher 2002) and poor verbal communication (Powers 1996) which can have a detrimental effect on employment opportunities in the long term (Davis & Mencher 2002). A study of 344 deaf pupils concluded that the severity of deafness was closely related to speech communication ability and social integration with hearing friends (Powers 1996). In view of the effect that deafness can have on children and their families, it is important to have information about prevalence and aetiology in the population.

The older the child when a congenital hearing loss is diagnosed, the greater are the chances of severe delay in language development (McFarland & Simmons 1980). The most important period for language development of a child is from birth to the age of 2.5 years (Moore *et al.* 1991, Egeli *et al.* 2003). Thus it has been believed for long time that earlier identification of hearing impairment must lead to better outcomes, and there is now reliable evidence that this is the case for communication development, educational achievement and quality of life (Robinshaw 1995, Yoshinaga-Itano *et al.* 2000, Moeller 2000). In a study of 112 children with deafness who were enrolled in a comprehensive intervention programme, vocabulary skills at 5 years were evaluated (Moeller 2000). A significant negative correlation was found between age of intervention and language development at 5 years. Children with earliest intervention (11 months) had significantly

better vocabulary than children with late intervention. Also in this study children with early intervention scored as well as the hearing children regardless of the severity of deafness. In a longitudinal study (Robinshaw 1995), 5 children with severe to profound deafness who had been wearing hearing aids from 3 to 6 months of age were studied up to 21 months of age. The authors concluded that children using hearing aids by 6 months of age acquired vocal communication and language skills at an age comparable with their normal hearing counterparts. This study also emphasised that reduced auditory stimulation for periods of only 3 to 6 months could delay the normal course of language acquisition. In another study (Yoshinaga-Itano *et al.* 2000), 50 children (25 matched pairs born in hospitals with and without newborn hearing screening) with deafness were analysed with regards to their speech and language development. It was found that children born in hospitals which had newborn hearing screening performed much better in both language and vocabulary than those who were born in hospitals that did not. Most authors agree that early identification and rehabilitation is better.

Methods of early detection of hearing loss

However, in spite of this evidence, even until quite recently, the mean age of detection of hearing impairment was about 24 to 33 months according to a number of published studies (Sarno & Clemis 1980, Newton 1985, Naarden *et al.* 1999). In the study of 111 children in UK the mean age at diagnosis of hearing loss was 23.5 months (Newton 1985). In the US the mean age of confirmation of hearing loss was reported as 28 months

(Sarno & Clemis 1980), and 33 months in another study of 173 children (Naarden *et al.* 1999).

Various screening and surveillance programmes have been introduced in order to try to reduce the age of identification of hearing impairment i.e. the health visitor distraction test (Haggard *et al.* 1992, McCormick 1993) and the targeted and universal neonatal screening (Stevens *et al.* 1991, Watkin 1996). The age of identification via the health visitor distraction test varied from 12 to 20 months with poor sensitivity and specificity (Davis *et al.* 1997). Protocols for screening newborns for hearing impairment, which were based on specified high risk criteria, usually identified only about half of all infants with congenital hearing loss.

Three key factors were included as criteria for targeting children (Stevens & Parker 2002):

- History of admission to Neonatal Intensive care unit for more than 48 hours
- Family history of early childhood deafness
- Syndrome associated with hearing impairment

It was estimated that 50% of all children with bilateral hearing impairment >50db had one or more of these three factors.

However it was proposed that Neonatal screening using otoacoustic emissions has higher sensitivity and higher specificity compared with other methods for hearing screening.

Otoacoustic emissions (OAE) originate in the cochlea and are due to the biomechanical process associated with normal hearing. OAEs can be recorded only when a region of

normal cochlear function is present and their absence could be due to presence of outer or middle ear disease or poor recording conditions or cochlear deafness. OAEs are recorded by a probe placed in the ear canal which has a loudspeaker to generate the sound stimulus and a microphone to record the sound in the ear canal. OAEs are classified into Transient evoked otoacoustic emission (TEOAE) and Distortion product otoacoustic emission (DPOAE). TEOAE is a response to a short transient sound. For DPOAE two sound stimuli at different frequencies are delivered. OAEs are not normally present when the hearing loss is more than 30dB (Stevens & Parker 2002).

Universal Neonatal Hearing Screening (now called Newborn Hearing Screening (Davis *et al.* 1997)) has now been introduced throughout UK (Davis *et al.* 2001b, NHS Executive 2004) because of its potential to reduce the age of confirmation of hearing loss (Dalzell *et al.* 2000). Data from the screening and assessments is kept nationally on a database integrated with other child services. This will help in monitoring the development of hearing loss at a later date and also to look at the effectiveness of the screening programme. The universal programmes implemented have given yield of expected prevalence (1-1.3 per 1000), with a median identification age for the children screened of about 2 months (Davis *et al.* 1997).

Screening for hearing loss in newborns has been shown to be cost effective and efficient (Stevens *et al.* 1998, Davis *et al.* 2001a). It has been reported to have a sensitivity of 80-90%, false positive rate of less than 2% (Yoshinaga-Itano *et al.* 2000, Kennedy *et al.* 2000) and a positive predictive value of 17%. The proposed costs of such screening in the United Kingdom are much lower than costs of the previous infant distraction screen test

(Pressman *et al.* 1999). It was recommended by Davis *et al.* that a programme based on universal neonatal screening followed at 7 months by a targeted screen using an infant distraction test is a good option for picking up children with deafness early (Davis *et al.* 1997).

1.1 Hearing Impairment

1.1.1 Epidemiology of Hearing Impairment

Epidemiological information can be used to plan and evaluate strategies to prevent morbidity and to effectively manage those in whom a disorder has already developed and the same can be said for hearing loss.

A number of studies have estimated the prevalence rates of permanent hearing impairment in children to be 1 to 2 per 1000 live births (Mäki-Torkko *et al.* 1998, Naarden *et al.* 1999, Uus & Davis 2000, Fortnum *et al.* 2001, Fortnum 2003). The range of severity is wide and for an individual may fluctuate over time. Severity may not be symmetrical for the two ears. The hearing impairment may develop progressively over time or it may be worsened temporarily by other causes (Fortnum 2003).

In a nationwide study, Fortnum *et al.* ascertained 17,160 children from all the postcode areas in UK (Fortnum *et al.* 2001). It was concluded that the prevalence of confirmed childhood deafness increases up to the age of 9 years to a level higher than estimated previously. In practical terms, for 1 hearing impaired per 1000 live births recognised by neonatal hearing screening, 50-90% more children are diagnosed with deafness by 9 years of age. This study was large and covered entire UK. As a result of the

comprehensive ascertainment of their cases, prevalence figures from this study (Fortnum *et al.* 2001) have been used for comparison with the data in this thesis.

1.1.2 Causes of Deafness

- Knowing the causes of deafness is important as it provides a more logical approach to management of these children and may help to organize preventive measures in the future (Janzen & Schaefer 1984).

For this study we have classified the causes of deafness into genetic, acquired and unknown. However, ascribing the cause to one of these categories is not always straightforward. The study examining the causes of hearing loss in children in Greater Manchester has highlighted the problems in establishing the cause of hearing loss in a child (Newton 1985). There can be multiple coexisting factors causing deafness in children. Late diagnosis of hearing loss reduces the chances of confirming hearing loss which is due to intrauterine causes and in spite of the difficulties, the clinician is expected to reach some conclusion regarding the cause of deafness.

It is estimated that approximately 50% of prelingual hearing loss is caused by genetic factors (Marazita *et al.* 1993), 25% are due to environmental factors (prematurity, infections, trauma and ototoxicity) and the cause is unknown in the rest 25% of cases. Even in the unknown group, most of the causes are assumed to be genetic in origin. Genetic causes account for the largest proportion of cases of prelingual hearing loss (Schrijver 2004).

The main causes of acquired/environmental hearing loss are pre or post-natal acquired infections, pre or post-natal drug therapy and other perinatal factors like prematurity, hypoxia, hyperbilirubinemia, neonatal sepsis and low birth weight. Infections acquired in the pre-natal period that can cause sensorineural hearing loss include rubella (Martin *et al.* 1981, Newton 1985, Parving & Hauch 1994), toxoplasmosis and cytomegalovirus (Newton 1985, Davis & Mencher 2002). The most common post-natal infection causing hearing loss is meningitis (Davis *et al.* 1995, Davis & Mencher 2002).

1.1.3 Aetiological Investigations

Investigations to determine the aetiology of deafness start with a detailed prenatal, medical and family history. Risk factors for the hearing loss such as low birth weight, prematurity, low APGAR scores, admission to neonatal ICU and jaundice should be assessed. This should be followed by a detailed clinical examination to exclude features compatible with a syndrome or congenital infections (in neonates).

Specifically an ophthalmologic assessment should be requested. Ocular abnormalities such as cataract, retinal pigmentation and optic atrophy can be present in children with severe to profound hearing loss and their diagnosis may help in determining aetiology as well as being important for the child's management (Armitage *et al.* 1995).

Laboratory testing should be individualized and directed towards the suspected diagnosis. The tests may include IgM antibody assay in first weeks of life to rule out the possibility

of intrauterine infections, urine analysis in patients with possible Alport syndrome, thyroid function tests and Perchlorate test in suspected Pendred syndrome and evaluation of metabolic disorders (Hone & Smith 2002). An ECG should be done to assess the cQT interval if Jervell Lange Nielsen syndrome is suspected.

Patients with unexplained hearing loss should have a renal ultrasound and temporal bone imaging. Imaging should include a high resolution CT scan to assess the bony structures (presence of malformations of the labyrinth) or MRI to assess the acoustic nerve and the inner ear.

Genetic testing which currently involves analysis for *GJB2* mutations, should be offered to any child with permanent bilateral non-syndromic hearing loss of any severity as it is a common cause of recessive hearing loss in most of the populations (Bitner-Glindzicz 2002). Moreover it requires a simple blood sample and the screening is straightforward and economical. Less invasive samples such as buccal cells obtained with a swab or part of the blood spots used for newborn screening could also be used.

Preciado et al recommended that a sequential testing approach to determine aetiology would be more appropriate and more cost effective than the commonly followed simultaneous approach (Preciado *et al.* 2004). In their algorithm, all patients with hearing loss should first undergo detailed case history review, physical examination and a complete audiologic review. When the aetiology is not determined, patients with bilateral sensorineural hearing loss should first undergo *GJB2* screening. If no abnormality is

found on screening, then imaging is performed. Laboratory testing should be ordered only when mandated by clinical findings. When bilateral hearing loss is severe, ECG testing is indicated. In patients with unilateral hearing loss, only temporal bone imaging is required and *GJB2* screening and laboratory testing is not required. Ophthalmologic evaluation should be carried out in all children with sensorineural hearing loss to rule out a coexisting sensory impediment. In children with bilateral profound hearing loss and normal genetic testing and imaging, electroretinography can be a valuable diagnostic tool for Type 1 Usher's syndrome (type 1 Usher causes profound HL and vestibular failure so can just do ERG in those with profound HL and delayed motor milestones).

Figure 1: Algorithm for evaluation & management of sensorineural hearing loss in children(Preciado *et al.* 2004)

1.2 Genes causing deafness

1.2.1 Genes

Genes consist of coding sequences (exons), interrupted by non-coding sequences (introns). However the coding sequences in the human genome comprise only 2-3% of all the DNA in the cell nucleus and the remainder is non-coding DNA (Read 1996)

Genes, in particular the coding region of DNA, specify the sequence of amino acids in a particular protein. DNA consists of four bases, Adenine, Thymine, Cytosine and Guanine and every three (a codon) in a coding region, specify a particular amino acid. They are transcribed (synthesis of an RNA molecule containing the genetic message) from the DNA template, and then translated (synthesis of a polypeptide chain as per instructions in the genetic code of mRNA) by cellular ribosomes.

Mutations in a gene can cause a disease because either the mutated gene may lose its function and do nothing, or it may gain a harmful function. Mutations that result in loss of function normally cause recessive phenotypes, because usually cells can function on half the dose of the gene product. Sometimes they can cause dominant disease if the cells cannot function on half the product.

Genes may be mutated in a variety of ways. For example, deletion mutations result in deletion of part of, or the entire gene; within a coding sequence of DNA, single base substitutions may replace a codon for an amino acid with one for a stop codon (Nonsense mutation) or may result in one amino acid being replaced by another (Missense mutation); splice site mutations create or destroy signals for exon-intron splicing;

frameshift mutations are small insertions, deletions or splicing errors that change the way the continuous mRNA strand is read in triplet codons (Stracan & Read 1999).

Polymorphisms are non-disease causing mutations which occur usually with prevalence greater than 1-2% in a normal population.

Mutations may be described in two ways. One is to describe the amino acid substitution i.e. V95M, which means that Valine at position 95 of the protein has been replaced with Methionine. The second method is to describe the DNA change i.e. 35delG, which means deletion of a single guanine at nucleotide number 35 of the gene.

Deletions of the whole gene, nonsense mutations and frameshifts almost certainly destroy the gene function and are unlikely to result in protein production. A missense mutation may or may not be pathogenic according to whether it affects a part of the protein which is functionally important. Amino acid substitutions are more likely to affect function if they are very different (acidic by a basic amino acid or amino acids of different sizes). A sequence change in a disease gene that is present in an affected patient and not in the unaffected parents is very likely to be pathogenic and is called *de novo* mutation (Strachan & Read 1999).

1.2.2 Genetic hearing impairment

Of the hearing losses attributable to genetic causes, approximately 70% are classified as non-syndromic and 30% as syndromic (Gorlin *et al.* 1995). Non-syndromic deafness is not associated with other physical features. On the other hand, syndromic deafness is characterised by features involving other systems running together with hearing loss, for example retinitis pigmentosa in Usher syndrome, craniofacial dysmorphism in Treacher

Collins syndrome, goitre in Pendred syndrome, long QT interval in Jervell and Lange-Nielsen syndrome and nephritis in Alport syndrome. In syndromic hearing loss it is important to make a correct diagnosis so as to monitor the individual and family for known complications of that syndrome. It is also important for genetic counselling that a correct diagnosis is made clinically or on molecular testing.

Non-syndromic hearing impairment can be further subdivided by the mode of inheritance. In the majority of the non-syndromic cases, approximately 80% show autosomal recessive inheritance, 20% are autosomal dominant, 1% are X linked and <1% are due to mitochondrial mutations (Morton 1991). Most non-syndromic autosomal recessive hearing impairment causes a prelingual hearing loss, which is usually severe to profound and not associated with any abnormal radiological findings. The dominant hearing loss is much more variable. Most non-syndromic autosomal dominant hearing loss is post lingual in onset and usually begins before 20 years of age, but it can be earlier ie. prelingual or can be much later in onset.

Remarkable progress has been made in the identification of the molecular basis of hearing loss in the past decade. To date 133 loci have been reported for non-syndromic hearing loss (54-autosomal dominant, 68-autosomal recessive, 8-X linked, 2-modifier loci and 1-Y linked locus). Of these loci, 45 of the genes for non-syndromic deafness have been identified and characterized (23-autosomal recessive, 21-autosomal dominant, 1-X linked) ([http: & webhost.ua.ac.be/hhh](http://webhost.ua.ac.be/hhh) 2005). These genes encode a wide variety of molecules including structural proteins eg motor proteins (*MYO7A*, *MYO6*, *MYO15*), ion

channels (*KCNQ1*, *KCNQ4*), transporters (*SLC26A4*) and gap junction proteins (*GJB2*, *GJB6*).

Table 1: Autosomal Recessive genes identified to date (Adapted from Hereditary hearing loss homepage (<http://webhost.ua.ac.be/hhh>))

Table 2: Autosomal Dominant genes identified to date (Adapted from Hereditary hearing loss homepage (<http://webhost.ua.ac.be/hhh>))

Table 3: X linked genes identified to date (Adapted from Hereditary hearing loss homepage (<http://webhost.ua.ac.be/hhh>))

However mutations in the gap junction gene, *GJB2*, encoding the Connexin26 protein are the most common cause of genetic deafness. They have been shown to account for a significant proportion of recessive non-syndromic deafness in most populations studied, and account for almost 50% of clearly recessive cases in Western and Southern Europe and between 10% and 37% of 'sporadic' cases (Estivill *et al.* 1998, Lench *et al.* 1998).

1.3 Molecular architecture of Inner Ear

The inner ear is the sensory organ for hearing and balance. It consists of the bony and membranous labyrinths. The bony labyrinth is divided into the vestibule, cochlea and semicircular canals (Figure 2). The fluid inside the bony labyrinth is perilymph which has high Na^+ and low K^+ concentration (like extracellular fluid) and inside the membranous labyrinth is endolymph, which has high concentration of K^+ and low Na^+ . The border between these fluids is at the level of epithelial cells that surround the endolymphatic spaces. It is essential to maintain the permeability of this barrier for the inner ear function. The endolymphatic compartment is surrounded by three types of epithelium: sensory epithelium, ion transporting epithelium and relatively unspecialised epithelia (Forge & Wright 2002).

Figure 2: Section of Inner Ear (Adapted from pages.cpsc.ucalgary.ca)

The sensory epithelium of the cochlea is the organ of Corti and that of the vestibular system are the maculae of the utricle and saccule and cristae of the semicircular canals. The sensory epithelium is composed of sensory hair cells and supporting cells (Figure 3). It is covered by the tectorial membrane in the cochlea, the otolithic membranes of the macular organs and the cupulae of the cristae. Hair cells are transducers converting a mechanical stimulus into an electric signal. Deflection of the hair cells caused by either sound waves (cochlea) or changes in head position (vestibule) modulates the flow of K^+ from endolymph through the hair cells and that excites the hair cell activity.



Figure 3: Section of Organ of Corti (Adapted from ourworld.compuserve.com)

The stria vascularis of the cochlea and the dark cell regions of the vestibular system are responsible for the ion transport necessary to maintain the endolymph composition.

The other less specialised epithelia, Reissner's membrane in the cochlea and the epithelium of the roof of the saccule, utricle and ampullae of the semicircular canals also form barriers separating the fluid spaces.

1.3.1 Hair cells

The hair bundle consists of rows of stereocilia and a single kinocilium located behind the row of longest stereocilia (Figure 4). Stereocilia consist of a core consisting of the cytoskeletal protein, actin, which is arranged in parallel bundles cross linked by fimbrin (Flock *et al.* 1982) and espin (Zheng *et al.* 2000). The espin gene is mutated in the *Jerker* mice, who are deaf and show vestibular disorder (Zheng *et al.* 2000). This shows the

importance of actin bundling and maintenance of stereociliary rigidity for hair cell function.



Figure 4: Structure of Hair cells (Adapted from vestibular.wustl.edu)

The cuticular plate is a platform that supports the stereocilia, and consists of a meshwork of actin filaments. Some actin filaments descend from the stereocilia into the cuticular plate as a rootlet and crosslink with the actin meshwork. The cuticular plate also contains spectrin (Yilkosky *et al.* 1992), which cross links between actin filaments and tropomyosin, which binds around actin (Forge & Wright 2002). Various members of the myosin family of motor proteins, types 1c, 6, 7a and 15 are also localised in the cuticular plate and stereocilia (Figure 5). Mutations in genes for different myosins cause hearing loss in humans (*MYO6*, *MYO7A*, *MYO15*). Experimentally it has been proven that those



Figure 5: Stereocilia showing Myosin distribution (Adapted from www.bms.ed.ac.uk)

genes for myosins 6, 7a or 15 all have hearing loss, balance disorders and abnormalities in the stereociliary bundles (Steel & Kros 2001). In the Snell's *Waltzer* mouse mutant where myosin 6 is defective, stereocilia are fused and greatly lengthened (Self *et al.* 1999). In the *Shaker 2* mice with myosin 15 mutation, stereocilia are greatly reduced in height (Probst *et al.* 1998). In *Shaker 1* mice with myosin 7a gene mutations, the stereocilia are separated (ie. splayed) apart from each other.

There are three different types of lateral links between stereocilia. The lateral links may play a role in holding the bundle of stereocilia together to stabilise it. These lateral links

may be composed of protein, cadherin 23. Mutations in cadherin genes can cause hearing loss and balance disorders. In Usher syndrome type 1F the protocadherin Pcdh 15 is defective, which is also seen in Ames *Waltzer* mouse mutant. In Usher syndrome type 1D in humans and some non-syndromic deafness, cadherin 23 is defective, which is also defective in *Waltzer* mouse strain. These proteins may form complexes that maintain the lateral tension between stereocilia (Figure 6).

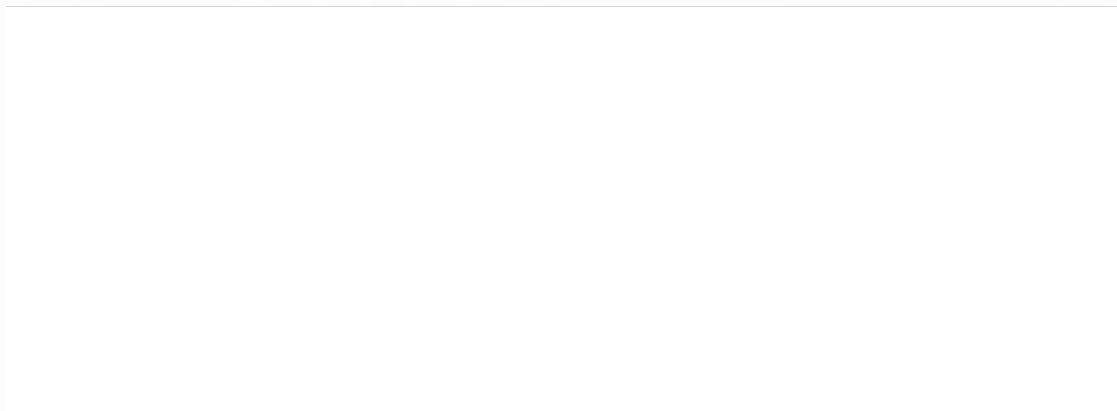


Figure 6: Stereocilia showing tiplinks (Adapted from www.ks.iuc.edu)

Supporting cells provide mechanical support to the epithelium and the hair cells. These supporting cells contain the β form of actin, intermediate filaments, different isoforms of cytokeratins (Kuijpers *et al.* 1991) and vimentin (Schulte & Adams 1989, Kuijpers *et al.* 1991).

1.3.2 Potassium recycling in the cochlea

In the cochlea, the stereocilia are surrounded by the endolymph, which has high potassium concentration. When stereocilia are deflected, K^+ ions from the endolymph flow through the channels in hair cells and result in depolarisation. As a result of this depolarisation the inner hair cells release neurotransmitter at synaptic junctions with the

afferent neurons. The outer hair cells fine tune the response to sound frequencies. The recycling of the K^+ ions (Figure 7) from the depolarised hair cells into the endolymph is an active ATP dependent process (Battey, Jr. 2000).



Figure 7: Potassium recycling in the cochlea (Adapted from www.ncbi.nlm.nih.gov)

A number of ion channels have been identified in the inner ear. Mutations in the potassium channel gene *KCNQ4* result in a dominant form of progressive hearing loss (Kubisch *et al.* 1999). This K^+ channel is important for transporting ions out of hair cells. It is postulated that after the potassium ions leave the hair cells, they pass through a network of gap junctions between supporting cells and fibrocytes in the cochlea on to the marginal cells of the stria vascularis, which secretes potassium rich endolymph. A sodium-potassium-chloride co-transporter protein encoded by *SLC24A2* helps to pump K^+ ions into the marginal cells of stria vascularis, which secretes endolymph. Those mice with mutation in this gene have profound deafness as no endolymph is produced (Dixon *et al.* 1999). Layers of stria vascularis contain a number of specialised channels and

pumps responsible for K^+ transport across it. The channels at the secreting surface of the marginal cells are made of proteins, encoded by the genes *KCNQ1* (previously known as *KvLQT1*) and *KCNE1* (previously known as *ISK*). Humans with mutations in the genes *KCNQ1* (Neyroud *et al.* 1997) or *KCNE1* (Tyson *et al.* 1997) suffer from Jervell and Lange-Neilsen syndrome. The mice with non functional *KCNE1* gene have profound deafness and vestibular hypofunction as no endolymph is produced (Vetter *et al.* 1996). Inactivation of *KCNQ1* gene in mice also results in profound deafness and vestibular abnormality (Lee *et al.* 2000).

1.3.3 Gap Junctions

Gap junctions (Figure 8) are sites of direct communication between adjacent cells. These are found both in invertebrates i.e. nematodes, echinoderms, ascidians, molluscs, arthropods and vertebrates i.e. frogs, chickens, rodents and humans (Wei *et al.* 2004). In invertebrates the gap junctions are encoded by the gene family known as innexins (Phelan & Starich 2001) and in the vertebrates, by the gene family connexins (Willecke *et al.* 2002). The gap junctions are present between supporting cells, but there are no gap junctions between hair cells. They are involved in several cellular functions including cell growth, differentiation, reaction to signals, synchronisation of activity in excitable tissues and homeostasis (Richard 2003).

1.3.3.1 Structure of gap junctions

Gap junctions are formed of proteins called Connexins. All connexins have a common architecture. Six connexins form a hemi channel called a connexon (Figure 7) and the connexons of two adjacent cells join together to form the communication pathway between the cells called the gap junction (Kumar & Gilula 1996, Yeager 1998),. Each gap junction contains several thousand connexons (Forge & Wright 2002).

The connexon channels allow the passage of ions, metabolites up to 1.2kDa in size and second messengers (Kumar & Gilula 1996). The gap junctions provide a means to remove the K^+ ions from the intercellular spaces to maintain K^+ homeostasis. The opening of gap junctions depends on calcium concentration, pH, transjunctional membrane potential and protein phosphorylation (Harris 2001).

Figure 8: Structure of gap junctions (Adapted from www.lrz-muenchen.de)

1.4 Connexins

Twenty one connexin genes have been identified in the human genome

(<http://www.ncbi.nlm.nih.gov/>) and twenty in the mouse genome. Most of the connexin genes have a common structure (Figure 9). They consist of a first exon containing the 5' untranslated region (UTR), followed by an intron and a second exon containing the remaining 5'-UTR, the coding sequence and the 3'-UTR (Willecke *et al.* 2002).



Figure 9: Structure of Connexin genes

The connexins are named using two different nomenclature systems i.e. by molecular mass or based on sequence. For example the 43kDa connexin protein is referred to as either connexin 43 or α_1 Connexin and Connexin 32 is also called β_1 connexin. The connexin proteins have been subdivided into three subgroups Connexin α , β or γ . Sometimes a hybrid nomenclature can be used such that Connexin 43 or α_1 Connexin is called Connexin43 α_1 (Wei *et al.* 2004).

Six connexins oligomerise to form a connexon, which represents half an intercellular channel. The properties of a gap junction depend on its constituent connexons. The gap junctions in a cell can range from few to over 10^5 , resulting in a uniform phenotype to the cells (Simon & Goodenough 1998).

Cells and tissues can express more than one connexin isotype. A connexon composed of six identical subunits is called homomeric and if composed of more than one connexin isotype is called heteromeric (Figure 10).

Two identical connexons can form a homotypic channel (Figure 9). If the channel is formed by two connexons having different connexin isotypes, it is called a heterotypic channel.

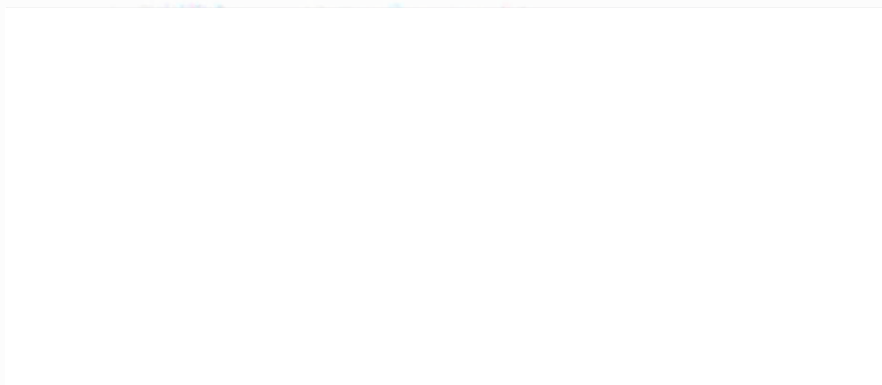


Figure 10: Composition of Connexons (Adapted from www.kent.ac.uk)

1.4.1 Connexins in human disease

Connexins are proteins that are present ubiquitously. They have been implicated in pathogenesis of diseases in the epithelial system, nervous system, eye and ear (Chang *et*

al. 2003). The diversity of diseases caused highlights the importance of connexins in different tissues and their functions. Different mutations in the same connexins can cause different tissue-specific diseases (*GJB3* mutations cause both erythroderma variabilis and autosomal dominant hearing loss). Also similar mutations in two different connexins can cause a single disease (Both *GJA3* and *GJA8* mutations cause cataract). Mutations in the genes of the *GJB* family causes epithelial disorders such as erythroderma variabilis (*GJB3*, *GJB4*), palmoplantar keratoderma (*GJB2*), hidrotic ectodermal dysplasia (*GJB6*) and Vohwinkle syndrome (*GJB2*); the neurological disorder Charcot Marie Tooth disease(*GJB1*) and eye disorders such as cataract (*GJA3,GJA8*) (Chang *et al.* 2003).

Connexins expressed in the inner ear are Connexin 26, 30, 31 and 32 and mutations in these genes result in syndromic and non-syndromic deafness (Kelsell *et al.* 2001).

Connexin gap junctions affect the ionic homeostasis of the cochlear epithelial cells and potassium recirculation from the hair cells to the endolymph (Kelsell *et al.* 1997,Saez *et al.* 2003,Chang *et al.* 2003). Impairment of normal ion channel function results in hair cell death and leads to sensorineural hearing loss (Rabionet *et al.* 2000,Saez *et al.* 2003).

1.4.2 Connexin 26

The Gap Junction Beta 2 gene, *GJB2* gene encodes for the protein Connexin 26 (Kelsell *et al.* 1997). Mutations in the *GJB2* gene are a common cause of deafness in many populations (Morell *et al.* 1998,Estivill *et al.* 1998,Cohn *et al.* 1999). In 1994 Guilford *et al.* identified a non-syndromic recessive deafness locus, DFNB1, at chromosome 13q12 (Guilford *et al.* 1994). In 1997, mutations at this locus were identified to be mutations in the *GJB2* gene (Kelsell *et al.* 1997), resulting in deafness. Connexin 26 deafness is now

recognised as the most common genetic cause of congenital deafness in many countries accounting for up to 50% of recessive deafness (Green *et al.* 2003).

The protein Connexin 26 is highly expressed in epithelial supporting cells of the mammalian cochlea. In the rat cochlea Connexin 26 is expressed mainly in two groups of cells, non-sensory epithelial cells and connective tissue cells. The first group i.e. epithelial cells, include interdental cells of spiral limbus, inner and outer sulcus cells, sensory supporting cells and cells within the root process of the spiral ligament. The second group i.e. the connective tissue cells include fibrocytes within the spiral ligament and spiral limbus, basal and intermediate cells of stria vascularis and mesenchymal cells which line the scala vestibule (Kikuchi *et al.* 1995). Expression of Connexin 26 in the vestibular labyrinth is similar. Mutations in *GJB2* cause deafness by altering Connexin 26 function within the inner ear (Green *et al.* 2003).

1.4.2.1 Mutations in *GJB2* (Genotype)

Approximately 110 non-syndromic deafness mutations have been identified in the *GJB2* gene. Of these 89 are recessive, 8 are dominant and 11 are of unknown significance. Some mutations are highly prevalent in particular populations (Roux *et al.* 2004). In some countries where mutational spectrum is limited, specific testing for certain mutations will identify most cases. The common mutations identified in *GJB2* have been the frameshift deletions 35delG, 167delT and 235delC and the large 342Kb deletion (Green *et al.* 2003).

There is marked ethnic variation in mutations found. In Caucasians the 35delG mutation, accounts for the majority of *GJB2* mediated hearing loss (Denoyelle *et al.* 1997, Sobe *et al.* 2000, Rothrock *et al.* 2003). The common mutations seen in other populations are- 167delT in the Ashkenazi Jewish population (Lerer *et al.* 2000, Sobe *et al.* 2000), 235delC in the East Asian population (Kudo *et al.* 2001, Wang *et al.* 2002), R143W in the African population (Brobby *et al.* 1998, Hamelmann *et al.* 2001) and W24X in the Indian subcontinent (Rickard *et al.* 2001).

The most common mutation in the *GJB2* gene is 35delG i.e. deletion of a single guanine from a series of six guanines. This results in a frameshift with premature termination of the protein. This mutation on its own accounts for nearly two thirds of the identified mutations in Caucasians. 35delG has been shown to be the most common mutation in patients with autosomal recessive sensorineural deafness from Australia, France, Israel, Italy, Lebanon, Morocco, New Zealand, Spain, Tunisia, UK and USA with relative frequencies ranging from 28% to 63% (Denoyelle *et al.* 1997, Scott *et al.* 1998, Estivill *et al.* 1998, Lench *et al.* 1998). The reason for this mutation being so common was earlier suggested to be due to the fact that 35delG is located in a hypermutable region (Denoyelle *et al.* 1997). As the prevalence of 35delG varies between different ethnic groups, an alternative to the mutation hot spot theory was proposed which was that the high frequency of this variant results from a common founder living 10000 years ago (Van Laer *et al.* 2001). 35delG is now accepted as a founder mutation.

The 167delT is the second most common *GJB2* mutation described and is highly prevalent in the Ashkenazi Jewish population (Sobe *et al.* 2000). This mutation also leads to a frameshift and premature truncation. Sobe *et al.* in their study of 75 Jewish hearing impaired subjects from Israel, have reported *GJB2* mutations in 29 patients (38.7%) (Sobe *et al.* 2000). Of these, 20 had the 35delG mutation (11 homozygous, 5 compound heterozygote with 167delT, 4-second mutation not found) and 13 had the 167delT mutation (8 homozygotes and 5 compound heterozygotes with 35delG) and 1 patient had a novel *GJB2* deletion/insertion mutation (51del12insA).

W24X (Tryptophan mutated to stop codon) is an inactivating mutation which was originally reported by Kelsell in two unrelated Pakistani families with profound deafness (Kelsell *et al.* 1997). In addition the mutation has been found to be common in Indian families (Scott *et al.* 1998, Rickard *et al.* 2001, Ghosh *et al.* 2004) and in one Thai patient (Kudo *et al.* 2001) which suggests that this mutation originates from the Asian population.

In a study on 11 families from a village in Ghana with high prevalence of hearing loss, Brobby identified R143W mutation as the cause of hearing loss in all of these families (Brobby *et al.* 1998). Another study of 365 unrelated individuals from all over Ghana found *GJB2* mutations in 63 patients (17%) (Hamelmann *et al.* 2001). Of these, 51 were homozygous for R143W mutation, 8 were heterozygous for R143W and 4 had other *GJB2* mutations. In this population from Ghana R143W contributed to 90% of all *GJB2* mutations seen.

Most *GJB2* mutations cause autosomal recessive non-syndromic hearing loss (DFNB1) although a few mutations have been identified in dominantly inherited hearing loss (DFNA3) (Morle *et al.* 2000, Loffler *et al.* 2001, Hamelmann *et al.* 2001). Dominant Connexin 26 deafness (DFNA3) has been identified in families with mutations, R184Q, W44C and C202F (Denoyelle *et al.* 1998, Morle *et al.* 2000, Hamelmann *et al.* 2001) and also R75W (Richard *et al.* 1998).

GJB2 mutations can also cause hearing loss in combination with *GJB6* (del Castillo *et al.* 2003). The *GJB6* gene encodes the protein Connexin 30, which can oligomerise with Connexin 26 in the same gap junction (Ahmad *et al.* 2003). See section 1.4.3 for further explanation of this mutation.

A more complete list of mutations is available at the connexin deafness homepage <http://www.crg.es/deafness>.

1.4.2.2 Connexin 26 deafness (Phenotype)

The severity of deafness in patients who have mutations in *GJB2* varies. In early studies Connexin 26 positive patients, profound deafness was found in 50%, severe in 30.2%, moderate in 18.1% and mild deafness in 1.7% patients (Steel 1998, Cohn *et al.* 1999, Mueller *et al.* 1999). However the severity of hearing loss can vary even amongst patients with the same mutations.

Almost all patients with deafness due to Connexin 26 have a flat or down sloping audiogram (Green *et al.* 2003). In few Connexin 26 patients, U shaped audiograms have been reported by Mueller (Mueller *et al.* 1999) . Mueller reported 4 of 31 patients with U shaped audiogram (mutations associated with U shaped audiogram not clearly stated). Selective low frequency hearing loss has not been identified in Connexin 26 deafness.

Most of the patients with Connexin 26 deafness have symmetrical hearing loss. Inter-auricular differences in the hearing thresholds have been reported in less than one quarter of the patients (Denoyelle *et al.* 1999, Cohn *et al.* 1999) with the difference usually less than 20dB.

Most individuals with Connexin 26 deafness have congenital hearing loss. In most of the patients, progression appears to be slow or nonexistent. However some authors have reported progression (Denoyelle *et al.* 1999, Cohn *et al.* 1999). Two of 16 children studied by Denoyelle et al over 10 years have shown progression (Denoyelle *et al.* 1999). Cohn et al have reported progression of hearing loss in 7 out of 30 patients over at least 6 years (Cohn *et al.* 1999). On the other hand none of the 12 patients in the study by Wilcox et al showed progression (Wilcox *et al.* 2000) and Mueller et al did not report any progression in the serial audiograms of 24 patients with *GJB2* mutations (Mueller *et al.* 1999). Patients with Connexin 26 hearing loss have loss of brainstem auditory evoked responses and otoacoustic emissions.

Connexin 26 deafness is usually audiologically quite stable. However in view of reports of progression, it is probably wise to follow up patients annually unless their hearing loss is already profound.

Patients with Connexin 26 positive do not have any bony abnormalities of the cochlea. Also these individuals have normal vestibular function and normal developmental motor milestones (Denoyelle *et al.* 1999, Green *et al.* 2003). In a study of 104 families (140 children) with sensorineural deafness, Connexin 26 deafness was present in 49% of families with prelingual deafness (Denoyelle *et al.* 1999). They concluded that Connexin 26 deafness varied from mild to profound, had a flat or sloping audiometric pattern and radiologically the inner ear was normal. Green *et al.* collected data from patients at University of Iowa & Arizona Connexin deafness consortium and concluded that Connexin related deafness was usually bilaterally symmetrical, congenital with no medical co-morbidities or vestibular abnormalities (Green *et al.* 2003).

1.4.2.3 Genotype Phenotype correlation

In non-syndromic deafness there seems to be some correlation between specific *GJB2* mutations and the phenotype on audiometric examination, although there is significant variability. Those mutations in the *GJB2* gene which completely inactivate the Connexin 26 synthesis (stop mutations or frameshifts) generally cause severe to profound hearing loss as compared to the non-inactivating mutations (missense mutations) or splice site mutations where some Connexin 26 protein is likely to be synthesised (Cryns *et al.* 2004, Snoeckx *et al.* 2005).

In a multicentre study of 277 unrelated patients with *GJB2* mutations from Belgium, Italy, Spain and United States it was found that 35delG homozygotes have significantly more severe hearing impairment compared with 35delG/non-35delG compound heterozygotes (Cryns *et al.* 2004). The authors also reported that two non-35delG mutations have even less hearing impairment i.e. non 35delG/non35delG. As a follow up study, *GJB2* genotype and phenotype in 1,531 patients with non-syndromic autosomal recessive hearing loss, from 16 countries were analysed (Snoeckx *et al.* 2005). It was found that hearing loss in patients with homozygous protein truncating mutations the hearing loss was significantly more severe than in patients with homozygous non-truncating mutations. It was also reported that hearing loss with 48 different genotypes was less severe than those who were homozygotes for 35delG. Other mutations such as M34T, V37I and L90P resulted in mild to moderate deafness. On the other hand 35delG/R143W and 35delG/delGJB6 had significantly more severe deafness than 35delG/35delG.

1.4.2.4 Cochlear implantation in Connexin 26

In patients with Connexin 26 deafness the neural structures are preserved, and these individuals do not have cognitive dysfunction. For these reasons it is expected that such patients would do well with cochlear implantation. It has been shown in some studies that cochlear implantation in children with Connexin 26 deafness has good results (Fukushima *et al.* 2002, Green *et al.* 2002, Matsushiro *et al.* 2002, Bauer *et al.* 2003) while others have reported no difference in the results (Cullen *et al.* 2004)

In another study 55 children who had been implanted before the age of 5 years were analysed (Bauer *et al.* 2003). Of these 22 children had *GJB2* related deafness and 33 were *GJB2* negative. It was seen that all children had benefit from cochlear implantation in the areas of speech production, speech perception and language. There was a significant positive difference in the cognitive and reading performance in children with identified *GJB2* mutations compared with those who were negative for *GJB2* mutations. The explanation for this is thought to be that *GJB2* mutations cause an isolated insult to the cochlea without damage to the auditory nerve or the central auditory system. Though the hearing loss in other children may be isolated and non-syndromic, there may have been subtle additional disabilities due to central effects.

In another study cochlear implantation in 8 *GJB2* positive patients of the 20 patients in the study group, had better results in terms of hearing as compared to other congenitally deaf cochlear implant patients and non-cochlear implant recipients (Green *et al.* 2002),. In a different study (Matsushiro *et al.* 2002), four patients with prelingual deafness with the 235delC mutation clearly benefited from cochlear implantation and performed better than implanted patients with no *GJB2* mutations. In Fukushima's study, three patients with *GJB2* related deafness had better speech as compared to four other children with non *GJB2* related deafness (Fukushima *et al.* 2002). Sinnathuray *et al.* compared 11 patients with *GJB2* mutations with 20 patients with *GJB2* unrelated deafness (Sinnathuray *et al.* 2004). They found that children with *GJB2* related deafness had equal or better speech discrimination on comparison with the other group with deafness of unknown cause.

Cullen compared 20 patients with *GJB2* mutations with 27 without mutations in *GJB2* (Cullen *et al.* 2004). In this study they found that the presence or absence of *GJB2* mutations did not have any significant impact on the speech performance at 12, 24 and 36 months after cochlear implantation.

1.4.3 Connexin 30

Connexin 30 is encoded by the gene *GJB6*. *GJB6* is a gene adjacent to *GJB2* on chromosome 13. Both of these genes are expressed in the cochlea and form multiunit gap junction channels. Mutations in either or both loci can cause deafness (del Castillo *et al.* 2002). Deafness manifests only when the patient has two mutations on opposite chromosomes. The most common mutation in *GJB6* is a 342kb deletion which causes non-syndromic hearing loss when homozygous or when present on the opposite allele of a *GJB2* mutation (del Castillo *et al.* 2002). This deletion truncates the *GJB6* gene but does not affect the *GJB2* structural gene. The deafness caused by *GJB6* deletion is explained by the hypothesis that there must be a regulatory element located upstream of *GJB2* and the deletion of this element would suppress the level of expression of *GJB2* enough to produce a phenotype of hearing impairment (Castillo *et al.* 2002). The hearing loss can range from mild to profound (Stevenson *et al.* 2003).

The 342kb deletion in *GJB6* gene is the second most common mutation causing hearing loss in Spanish population (Castillo *et al.* 2002).

1.5 Deafness in Bangladeshi children in East London

Bangladesh is a country in South Asia bounded by India from the north, east and west and Bay of Bengal and Burma from the south. Dhaka is the capital and largest city of Bangladesh. Bangladesh is divided into 6 divisions, all named after their respective capitals. These are Sylhet, Chittagong, Dhaka, Khulna, Rajshahi and Barisal.

The population of Bangladesh is nearly 150 million with about 40% of the population in 0-14 years age group, 57% in 15-64 years and 3% in 65 years and over. Apart from very small countries such as Singapore and Bahrain, Bangladesh is the most densely populated country in the world.

Despite sustained domestic and international efforts to improve economic and demographic prospects, Bangladesh remains a poor and overpopulated nation. Major impediments to growth include frequent cyclones and floods, inefficient state owned enterprises, a rapidly growing labour force that cannot be absorbed by agriculture, inefficient use of energy, insufficient power supplies and slow implementation of economic reforms (Sylhet Corp. 2005).

The population of Bangladesh is ethnically homogeneous, with Bengalis comprising 98% of the population. The vast majority of population (98%) speaks Bangla (sometimes called Bengali), the official language, and the remaining two percent are Urdu speaking. Most Bangladeshis (83%) are Muslims, but Hindus constitute a sizeable (16%) minority.

The dialects of Sylhet, Chittagong and Noakhali are strongly marked by Arab-Persian influences.

1.5.1 Sylhet

Sylhet is situated in the north eastern region of Bangladesh (Figure 11). It has a population of 0.7 million people and is called the tea granary of Bangladesh. It has over 150 tea gardens and also possesses the three largest tea gardens in the world with respect to dimension and production.

Figure 11: Map of Bangladesh showing Sylhet (Adapted from fr.wikipedia.org)

Sylhet belongs to a group of medium urban centres that have grown rapidly in last few years. The population density is estimated to be 2800 people per square mile. The health of the citizens of Sylhet is affected by lack of urban housing, air quality and lack of education and basic infrastructure.

1.5.2 The Bangladeshi population in East London

East London contains the highest number of Bangladeshis outside Bangladesh and the Bangladeshi population living in London is mainly concentrated in the boroughs of Tower Hamlets, Newham and Hackney. In Tower Hamlets, according to the 2001 census, over one third (33%) of the population of the borough is Bangladeshi, of whom half are under 20 years old. Almost all the Bangladeshi population living in this borough originates from the province of Sylhet and Tower Hamlets has the largest Sylhetti population in the world outside Bangladesh.

The population of Tower Hamlets is very interesting from the social and economic point of view. It is set to increase faster than the rest of London between now and 2011. Tower Hamlets has a much younger population than the rest of London and UK. It has the largest percentage of 20 to 34 year olds of any local authority in UK. 57% of the population are 15-44 years old compared with 41.5% for this age group for the country as a whole. The situation is reversed for 45-79 years old (Research & Scrutiny 2004).

Most of this difference between Tower Hamlets and the rest of London and the UK is due to the Bangladeshi population. The number of Bangladeshis under the age of 18 is almost double the proportion for all Londoners. This is a result of high fertility rates in this community. Most Bangladeshi children in London were born in UK while most adults were born in Bangladesh (GLA Data management and analysis group 2004).

Age profiles are very different for the two major ethnic communities in Tower Hamlets - White British and Bangladeshi. For the White British community, 40% are below the age of 30 and 12.5% are below 15 years of age. In the Bangladeshi community 70% are below 30 and 40% are below the age of 15 years.

According to the Indices of Deprivation, Tower Hamlets ranks as the second most deprived local authority in England (Research & Scrutiny 2004). The indices used include employment, health and disability, education skills and training, housing, living environment and crime (Office of National Statistics 2001). Tower Hamlets has a low life expectancy both for males and females as compared to national levels. The literacy rate amongst Bangladeshi women is low and very few of the women can communicate in English (Research & Scrutiny 2004).

Britain's Bangladeshi community lives with greater overcrowding than any other ethnic group (Kempson 1999). Nineteen percent of Bangladeshis live at a housing density of more than 1.5 persons per room, compared with 0.5% of the population as a whole (Kempson 1999).

Thus the demographics of population in Tower Hamlets make the borough exceptional in contrast to regional and national trends. This has an impact on delivery of local services in the future.

1.6 This study

In 1993, Vanniasagaram et al, in a 5 year review reported the prevalence of deafness in East London to be three times the national average (Vanniasagaram *et al.* 1993) . They also reported that the prevalence among Bangladeshi children living in Tower Hamlets in East London was about 6 times the national average. A comparison of the rates of deafness among Caucasian and Bangladeshi children in Tower Hamlets, showed the prevalence to be greater among the Bangladeshi children. This was attributed to high rates of consanguinity, although evidence for this was not presented.

1.6.1 Objectives of the study

In view of the expansion of the Bangladeshi population in East London within the last decade, the increase in knowledge of genetic causes of deafness with the possibility of molecular diagnosis and the advent of Newborn Hearing Screening, it was decided to review the prevalence and causes of deafness in this population in some detail. The objectives of this study were:

1. To determine the prevalence of deafness among the Bangladeshi population in 2005 and to compare this with national prevalence figures of Fortnum et al (Fortnum *et al.* 2001).
2. To review the causes of deafness generally.
3. To determine the genetic causes of deafness in this population:
 - i. To test for *GJB2* and determine its contribution to deafness

- ii. To identify families suitable for linkage mapping in order to identify other genes segregating in this population.
- 4. To review the attitudes of this population to hearing loss and to genetics.

2 Patients & Methods

The primary aims of this project were to determine the prevalence of deafness in the Bangladeshi population of East London and to determine causes of the deafness.

2.1.1 Ethical Committee approval

This project was registered with the Research and Development office at Great Ormond Street Hospital and the Institute of Child Health (R&D Registration number: 03CM16) by Mr Yogesh Bajaj. Ethical committee approval for this project was obtained from the Research Ethics Committee at Great Ormond Street Hospital & Institute of Child Health as well as City & Hackney NHS trusts in December 2003 for Mr Yogesh Bajaj, Dr Bitner-Glindzicz and Dr Sirimanna to conduct the project. This study entailed analysis of the patient records for all the patients, and recruitment of selected patients for further data collection and blood sampling for genetic analysis.

2.2 Patients

2.2.1 Patient ascertainment

In order to determine prevalence, as complete an ascertainment as possible was attempted. Details of children of Bangladeshi ethnic origin with hearing loss were obtained from various sources (Audiology database, Teachers of Deaf database, and individual Consultant's records). The records maintained by the audiology department at the Donald Winnicott Centre, of the children who have been issued with hearing aids in the past, were cross checked with the records of the Teachers of Deaf involved with these children.

The Donald Winnicot Centre (DWC) is a multidisciplinary community based clinic in Hackney which provides primary and secondary level health services including audiological services to the population in Tower Hamlets and Hackney districts. It is affiliated with Great Ormond Street Hospital for Children and Institute of Child Health, which provides tertiary level paediatric audiology services. All these patients were under the care of Consultant audiological physicians. The patients were seen at a research clinic in the audiology department at the Donald Winnicot Centre.

2.2.2 The study group

The Bangladeshi population living in East London is concentrated mainly in Tower Hamlets and Hackney health districts (Office of National Statistics 2001). The patients included in this study were all the Bangladeshi children on the records of Donald Winnicot Centre (DWC) with bilateral sensorineural hearing loss of more than 40 dB in the better hearing ear.

The Pure Tone threshold was calculated by taking the average of four frequencies -500, 1000, 2000 and 4000 Hz. The most recent audiogram was used to categorise the severity of hearing loss and the better hearing ear was used to classify the severity of hearing loss (as described in definitions section) (Stephens 2001). The shape of the audiograms were also recorded (Stephens 2001)

The data was collected from the patient notes (180) of all the children ascertained from different sources. Those children with conductive deafness or not actually of Bangladeshi origin were excluded from the study at this stage (34). Also patients with mild

sensorineural hearing loss i.e. less than 40 dB (8), and those with unilateral sensorineural hearing loss (4) were excluded from this study. In other words all children of Bangladeshi ethnic origin with bilateral sensorineural hearing loss more than 40dB in the better hearing ear were included in this study (Figure 12).

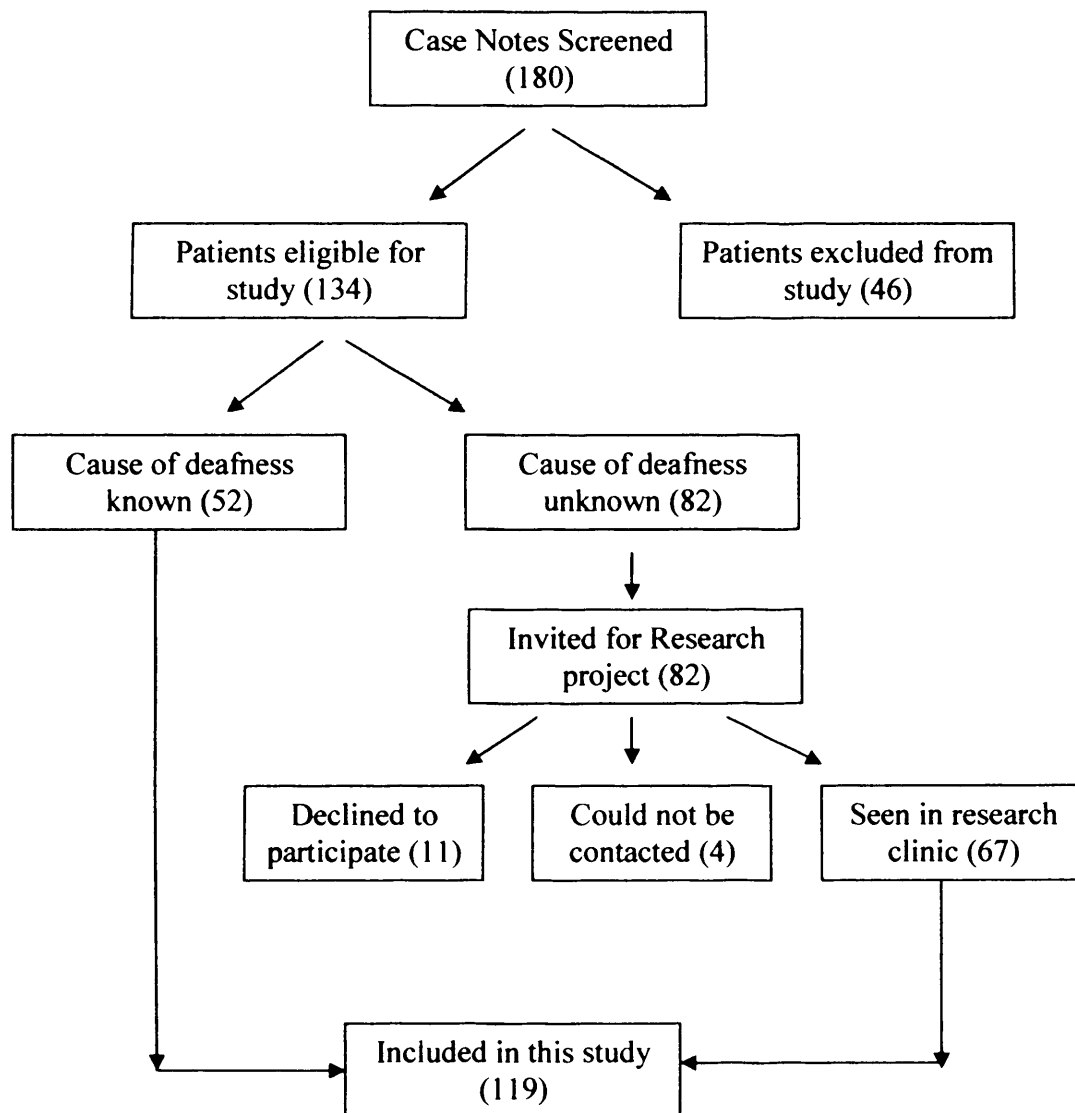


Figure 12: Patients selection for the study

2.3 Methods

2.3.1 Information about research

Information leaflets about the research project were prepared for parents and children by Mr Yogesh Bajaj in English, and translated into Bengali by professional translators. Sylheti, the dialect spoken by this Bangladeshi community does not have a written script and so further provision was made for parents who could not read English or Bengali and could only speak Sylheti. Audio tapes in Sylheti were recorded by a Sylheti speaking link worker, working with deaf children and their families (Ms Shabnam Sattar). The tapes were essentially translation of the information leaflet in the Sylheti dialect. The information leaflets in English and Bengali translation are attached as appendix 10.3. The audio CD with the Sylheti translation of the information leaflet is available on request.

2.3.2 Investigations

Most patients were regularly under the follow up of Consultant audiological physicians at Donald Winnicot Centre and Great Ormond Street Hospital. Many of the patients had already been investigated for their deafness. The tests included: full blood count, urea & electrolytes, thyroid function tests, ESR, urine analysis, ECG, ultrasound of kidneys, and CT scan of temporal bones. If the history had suggested teratogenic cause, the patients had been tested for common infectious diseases (toxoplasma, rubella, cytomegalovirus, herpes simplex, syphilis). A pre-printed list of investigations was used to request investigations for these patients at Great Ormond Street hospital. For most of the patients seen until 2-3 years ago, no genetic testing had been requested.

The serial hearing tests of these patients were recorded in the notes or were in the audiology records at Donald Winnicot Centre. The hearing had been assessed by pure tone audiometry in the older children and by sound field tests and Brainstem evoked response audiometry in the younger children.

2.3.3 Data Collection

The data collected included detailed history of hearing loss and the medical history, examination findings, audiological information on patients and parents and results of investigations. After the data collection at this stage patients were discussed at meetings with Dr Bitner-Glindzicz and Dr Sirimanna regarding inclusion into or exclusion from the study. Selected data were entered onto a Microsoft Access database designed by Mr Yogesh Bajaj for this purpose. At this stage, the patients were classified into two main groups- 'Cause of deafness known' and 'Cause of deafness unknown'.

2.3.4 Population Data

General demographic information about the Bangladeshi population in the UK was requested from the Research & Development office in Tower Hamlets and the Census office (Office of National Statistics 2001). Relevant information about the Bangladeshi population living in Tower Hamlets and Hackney had to be extracted from the available Census data. The relevant information is attached as appendix 10.6

2.3.5 Research Clinic

For those patients in whom the cause of deafness was not clear from the records or unknown (or non-syndromic deafness), families were invited to be seen in the research clinic by Mr Yogesh Bajaj specifically for this study. The invitation involved explaining

the research to the parents over the telephone in English or in Sylheti, if they did not understand English. This was followed up by written information in English and Bengali sent to these patients by post. Those who agreed to participate were given appointments to be seen in the research clinic at Donald Winnicott Centre.

All the patients were seen in the presence of Sylheti speaking liaison worker (Ms Parveen Quader), who knew the families well. The role of the Sylheti liaison worker has been vital for this project as the patients felt very comfortable in her presence. Written informed consent was obtained from the patient or the parents, in English or Bengali. For these patients and their families seen in the research clinic, detailed history and examination was carried out and blood samples were collected from the patient and the parents for genetic analysis. Blood samples were also taken from siblings if the parents were consanguineous. This was for possible future use for mapping studies.

2.3.6 Genetic analysis

The DNA was extracted at the North East Thames Regional Clinical Molecular Genetics laboratory under quality assured conditions and screened for mutations in *GJB2* by Ms Lucy Jenkins in the North East Thames Regional Clinical Molecular Genetics laboratory. All the samples were screened for mutations in the coding region (exon 2) and also for a splice site mutation (IVS1+1) and the 342kb deletion.

2.3.6.1 *GJB2* Analysis

Performed by Lucy Jenkins, Clinical Scientist at the Molecular Genetics laboratory.

2.3.6.1.1 PCR amplification

The *GJB2* gene was amplified by PCR in several fragments; the non-coding exon 1 including its splice site primers was amplified and the coding exon 2, which was amplified in three fragments 2A, 2B and 2C. After PCR, these reactions were performed spiked with a known wild type DNA and subject to DHPLC analysis.

Prior to loading onto the WAVE™ machine for DHPLC analysis, heteroduplex formation was carried out on the PCR products by denaturing at 95°C for 5 minutes followed by 1 minute cycles starting at 95°C and decreasing by 1°C per cycle for 46 cycles.

Heteroduplexed PCR products underwent DHPLC analysis at the following temperatures; exon 2 A at 61.8 °C, exon 2 B at 60.4 °C and 61.8 °C, and exon 2 C at 57.5 °C and 60.0 °C.

Table 4: Primers used for connexin 26 analysis.

	Primer	Primer Sequence	Size (bp)	Reference
Exon 1	1 F	5'TCCGTAAC TTTCCCAGTCTCCGAGG GAAGAG3'	360	(Kelsell <i>et al.</i> 1997)
	1 R	5'CCCAAGGACGTGTGTTGGTCCAGC CC3'		
Exon 2 A	CX26 AF	5'TCTTTTCCAGAGCAAACCGC3'	287	(Kelsell <i>et al.</i> 1997)
Exon 2 B	CX26 AR	5'GACACGAAGATCAGCTGCAG3'	410	In house
	CX26BF	5'CTGGTGCTACGATCACTAC3'		
	CX26BR	5'TTCCAGACACTGCAATCATG3'		
Exon 2 C	CX26CF	5'TATGTCATGTACGACGGCT3'	239	In house
	CX26CR	5'TCTAACAACTGGGCAATGC3'		

Table 5: PCR Conditions to amplify *GJB2* in three fragments for DHPLC analysis

	Exon 2 Fragment		
	A	B	C
PCR Product size (bp)	287	410	239
Reaction volume (dl)	30	30	30
Primer concentration (pmol / reaction)	5	5	5
Annealing temp. (°C)	63	62	62
Polymerase	Megamix W*	Platinum Taq**	Megamix W*
Magnesium Chloride	-	2mM	-
dNTP	-	200µM	-

* Megamix W (Microzone) contains magnesium (final concentration 2.5mM) and dNTPs (final concentration 200µM) but excludes BSA to allow DHPLC analysis.

** Invitrogen

2.3.6.1.2 Sequence analysis

Variants detected by DHPLC analysis were characterised by direct sequence analysis.

Fragment Exon 2 A was sequenced using the same primers used for PCR amplification, while fragments B and C were amplified and sequenced together as a 516 base pair product using CX26 BF and CX26CR. Bi-directional sequencing was carried out using Big Dye Terminator reactions followed by sodium acetate and ethanol precipitation then 70% ethanol wash. Sequencing products were analysed using an ABI 3100 or 3730 genetic analyser.

Exon 1 splice site mutation detection

Exon 1 and its exon-intron boundaries were amplified with primers. The splice mutation IVS1+1 removes a *Hph*I site and is therefore detected using restriction digest analysis of PCR products resolved on a 2% agarose gel.

2.3.7 Attitudes to Genetic testing

In the research clinic, families were also asked some general questions to assess their attitudes towards genetic testing. The parents and patients who agreed to participate in the research were asked their reasons for participation and about their attitudes to genetic testing, counselling and pre-natal diagnosis.

2.3.8 Follow up

For all the patients who were seen in the research clinic, letters were written to their General Practitioner and their primary audiology consultant informing them of the participation in the research project. Also these patients were sent letters to thank them for participating in the research. At regular intervals letters have been written to all these patients and parents informing them about the progress of the research. As the results of *GJB2* mutation screening become available, letters were written to the parents, their General Practitioners and the Consultants. Those patients who were found to be positive for mutations in *GJB2* were invited to come to genetic clinic to be seen by Dr Bitner-Glindzicz.

3 Prevalence of Deafness

3.1 Results

3.1.1 Families

One hundred and thirty four patients (115 families) were eligible to be included in this study. These patients were divided into two groups. Of these 11 patients (11 families) declined to participate and 4 patients (4 families) could not be contacted. Thus for the purpose of elucidating the cause of deafness 119 patients (100 families) were included. Of these 67 patients (53 families) had children with undetermined cause and 52 patients (47 families) had children with cause of their deafness diagnosed. Those patients in whom the cause of deafness was not known were invited to come to the research clinic.

3.1.2 Demographics of the study group

The patients in this study ranged from 9 months to 27 years of age. The average age at the time of recruitment in this project was 11 years 6 months, although most of the patients (121/134) were less than 18 years of age at the time of recruitment. In this study group there were 71 (52.9%) males and 63 (47.1%) female patients. All the subjects had parents or grandparents born in the province of Sylhet in Bangladesh. The duration of patients' hearing loss was in the range of 1 month to 26 years.

3.1.3 Prevalence of deafness in Bangladeshi children

The total population of Bangladeshis living in Tower Hamlets & Hackney districts in East London was 71,525 in 2001 (Office for national statistics 2001). Of these, there were 28,497 children less than 16 years as per 2001 census (Appendix 10.6).

In the course of this study, 134 deaf children with a permanent bilateral sensorineural hearing loss greater than 40dB in the better hearing ear, living in these two districts at the time of census in 2001, were ascertained from all sources and 110 of these children were aged up to 16 years of age (less than 16) in 2001. Thus the prevalence of deafness in Bangladeshi children in 2001 was at least 3.86 per 1000 (95%CI: 3.24, 4.47). This may be an underestimate as we may have incompletely ascertained some individuals, although all efforts have been made to trace these children using different sources.

3.1.3.1 Effect of Consanguinity

Vanniasagaram attributed the high prevalence of deafness in this community to consanguinity (Vanniasagaram *et al.* 1993). We wished to try and determine whether this was truly the case. There are no figures for consanguinity rates in Tower Hamlets as this data is neither collected during the census or locally by the Tower Hamlets authorities. Estimates of probable consanguinity rate therefore had to be made from published data (Durkin *et al.* 2000). Durkin *et al.* estimated the consanguinity in the urban Bangladeshi population in Bangladesh to be 10.6% and thus 10.6% was used to estimate the probable consanguinity rate among the parents of hearing children in Tower Hamlets. On removing 10.6% from 28,497 we are left with overall population of 25,809 children less than 16 years of age whose parents would not be consanguineous.

Among the children with deafness, 110 were less than 16 years of age in 2001, 40 children had consanguineous parents, leaving 70 deaf children who did not have consanguineous parents. On calculating the prevalence of deafness in these Bangladeshi children belonging to non-consanguineous families, the prevalence falls to 2.72 per 1000 (95%CI: 2.10, 3.34). The prevalence in this Bangladeshi population is still higher than the national figure of 1 to 2 per 1000 (95%CI: 2.02, 2.08) (Fortnum *et al.* 2001), so the excess can not be accounted for by consanguinity alone.

Table 4: Prevalence in British Bangladeshi children less than 16 yrs in 2001

Population	Prevalence	95% Confidence intervals
Whole population	3.86 per 1000	3.24 to 4.47 per 1000
Non-consanguineous population	2.72 per 1000	2.10 to 3.34 per 1000

Given the possibility that the estimate of background consanguinity in Tower Hamlets was taken to be 10.6%, it was important to determine whether errors in this estimate were likely to affect our calculations of prevalence of deafness. Figure 13 (Courtsey M Cortina-Borja, Senior Lecturer in Statistics, Institute of Child Health) shows that there are no substantial changes in the prevalence of deafness in non-consanguineous marriages with respect to possible values of the proportion of consanguineous marriages (between 5% and 20%) in the background Bangladeshi population; the prevalence estimates vary between 2.81% (95% confidence intervals 2.21 to 3.51) and 3.34% (95% confidence

intervals 2.63 to 4.17). The background prevalence of deafness in UK and its confidence intervals are shown for comparison (as calculated by Fortnum et al.)

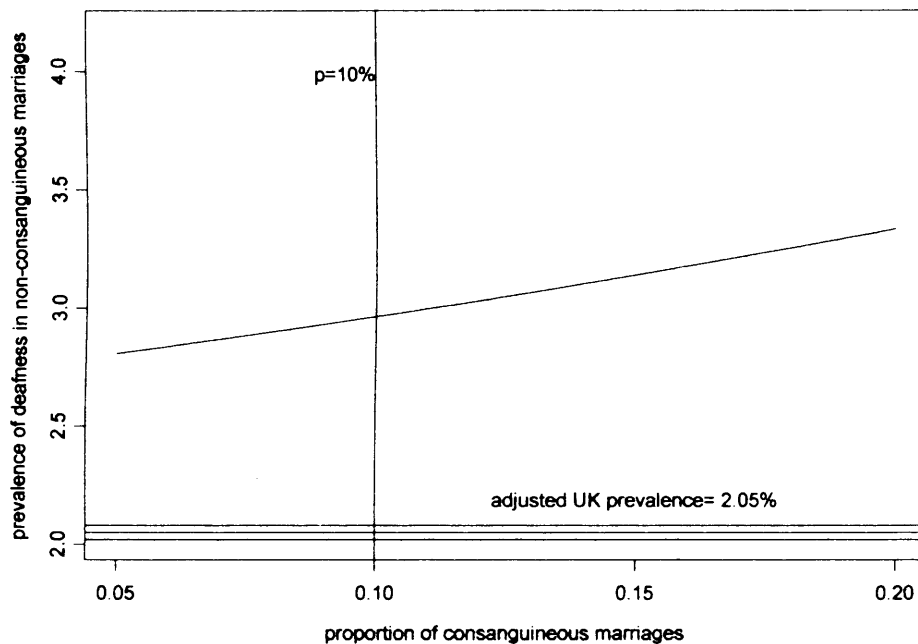


Figure 13: Prevalence of deafness vs consanguinity (The graph shows how the prevalence of deafness in non-consanguineous families would change with consanguinity from 5% to 20%. The solid line in the graph [marked p=10%] along y axis indicates the prevalence with 10% overall consanguinity)

3.1.4 Consanguinity in the study group

Information about consanguinity was available in 105 families, of which parents were consanguineous in 35 (33.3%). Of these, 26 parents were first cousins, 7 second cousins and 2 were third cousins.

Table 5: Consanguineous families: Total-35

Relationship between parents	Number of families	Percentage
First cousins	26	74.2%
Second cousins	7	20.0%
Third cousins	2	5.7%

3.2 Discussion

In the UK the prevalence of confirmed permanent childhood hearing impairment between 0 and 3 years is 0.91/1000 children (95% confidence intervals 0.85 to 0.98) rising to 1.65/1000 (1.62 to 1.68) for children aged between 9 and 16 years according to Fortnum *et al* (Fortnum *et al.* 2001, Fortnum 2003). Adjustments for under-ascertainment increased estimates to 1.07 (1.03 to 1.12) and 2.05 (2.02 to 2.08) respectively (Fortnum *et al.* 2001).

In this study the prevalence of deafness in children of Bangladeshi origin resident in East London up to 16 years of age was calculated to be at least 3.86 per 1000 which is 2.3 times the national average of 1.65 /1000. In Vanniasagaram's study, performed nearly 15 years ago, the prevalence of deafness in Tower Hamlets in East London was reported to be three times the national average (Vanniasagaram *et al.* 1993) and the higher prevalence among British Bangladeshi children was hypothesized to be secondary to high rates of consanguinity in this ethnic group although no data were presented. Higher prevalence of deafness in a number of different immigrant ethnic groups in the UK has been previously reported; Morton (Morton *et al.* 2002) reported a prevalence of deafness of 3.67/1000 children in a Pakistani population from South Derbyshire, and Naeem and Newton found a prevalence of 6.79 per thousand in Asian children aged 5-16 years compared to 2.1 per thousand for non-Asian children (using a hearing loss of 20dB or more, rather than 40dB used here for case ascertainment) (Naeem & Newton 1996). In these studies the excess of childhood deafness was also attributed to genetic causes secondary to the practice of consanguineous marriage.

It has been anecdotally known that cousin marriages were common in the Bangladeshi community. Durkin et al found that consanguinity was nearly 10% in the general urban Bangladeshi population (Durkin *et al.* 2000). The reason for high consanguinity is usually cited as arranged marriages, whereby parents arrange marriages amongst their relatives whom they know. The other reason cited is that if they are married to relatives, the family wealth stays within the extended family.

Amongst this whole study group of Bangladeshi children with hearing loss, the consanguinity was found to be nearly 34% amongst parents i.e. more or less 1 in every three families. Most of these parents (75%) were first cousins.

In the British Bangladeshi population our analysis suggests that consanguinity contributes to the raised prevalence of deafness. However removal of those children born of consanguineous marriages from the calculation does not reduce the prevalence to the national figure. It is likely therefore that environmental factors may also play a part in the high prevalence of deafness.

The environment of Tower Hamlets is unusual for many reasons; the borough of Tower Hamlets, where 33.4% of residents are of Bangladeshi origin (Office of National Statistics 2001), is ranked as the 2nd most deprived out of 384 in the country, has high unemployment rates and is overcrowded (10,462 people per square kilometer compared with 4679 for the region and 380 for England overall). The total fertility rate in Tower Hamlets is 1.84 compared to 1.64 for UK and Bangladeshis in general tend to have large

families. For example in London generally, 57% of Bangladeshi households have two or more dependent children compared with the London average of 17% and only 8% of Bangladeshi households are single person households (Research & Scrutiny 2004). It is possible that an environmental agent known to cause deafness, which is more prevalent where there is overcrowding and which associated with poorer socio-economic conditions might also account for some of this increase in prevalence.

Cytomegalovirus is now recognized as the most frequent cause of congenital infections in humans and is the leading acquired cause of congenital sensorineural hearing loss (Vallely *et al.* 2002). It occurs in approximately 1% of all newborns and hearing loss, which can range from mild to profound, is the most common neurological impairment associated with congenital CMV infection. As only 10% of infants with congenital CMV are symptomatic at birth it can be extremely difficult to diagnose in the majority of cases. Of the asymptomatic infants, 5% can later manifest symptoms of the disease including hearing loss. Another 10-15% of those asymptotically infected at birth can develop progressive hearing loss. This could represent the reactivation of the virus to an unknown stimulus (Lucas 2002). Thus, although speculative, it is possible that congenital CMV infection may account for some of the increase in prevalence of deafness in the Bangladeshi population of East London.

4 Causes of deafness

4.1 Classification

The causes of deafness were classified into three main groups: genetic, acquired (environmental) and unknown. The deafness was classified as genetic if it was syndromic or chromosomal or non-syndromic with a definitive inheritance pattern (dominant, recessive or X-linked). For those with genetic deafness, mode of inheritance was designated as autosomal recessive if more than one child in the family of normally hearing parents was deaf, or if parents stated that they were consanguineous and no other cause of deafness could be determined, or if the child was positive for *GJB2* mutations on subsequent testing.

4.1.1 Causes for the whole study population

The causes of deafness are shown in Table 6.

Table 6: Causes of deafness before *GJB2* testing, 119 patients (100 families)

	No. of patients	No of families	% of genetic	% of total
Genetic	68	50		57.1
Non-syndromic	38	24	55.9	
Dominant	1	1		
Recessive	37	23		
X-linked	0	0		
Syndromic	18	14	26.5	
Chromosomal	12	12	17.6	
Acquired	22	21		18.5
Unknown	29	29		24.3

We proceeded to test all children in whom the cause of deafness was unknown, for mutations in the *GJB2* gene (see Chapter 5 for more details). On analysing this same

group of 119 patients (100 families), the distribution changed to that shown in Table 7, which does not change the overall distribution of the causes of deafness to a large degree.

Table 7: Causes of deafness after *GJB2* testing: 119 patients (100 families)

	No. of patients	No of families	% of genetic	% of total
Genetic	71	53		59.6
Non-syndromic	41	27	57.7	
Dominant	1	1		
Recessive	40	26		
<i>GJB2</i> +	20	14		
X-linked	0	0		
Syndromic	18	14	25.3	
Chromosomal	12	12	16.9	
Acquired	22	21		18.5
Unknown	26	26		21.8

For the group as whole (119 patients/100 families) genetic causes appear to be the most common cause of deafness in the Bangladeshi population accounting for 59.6% of patients. Acquired causes were responsible for hearing loss in 18.5% patients and in 21.8% cases the cause of deafness was undetermined. Of the deafness due to genetic causes, 57.7% were non-syndromic, 25.3% syndromic and 16.9% were chromosomal. Amongst the 12 children who had hearing loss due to chromosomal causes, most (93.3%) of these children had Down syndrome and one child had Trisomy 8. In the 27 families with non-syndromic deafness nearly all patients (96.2%) had recessive deafness except 1 family who had dominant deafness.

4.1.2 Deafness associated with a syndrome

In the group with syndromic deafness (18 patients/14 families) the most common cause was an unknown syndrome in nearly one third of patients. These children had dysmorphic facies or other clinical features but no specific diagnosis could be assigned to them. The other syndromes associated with hearing loss found in this study are shown in Table 8. Consanguinity in the group of patients with an unknown syndrome was 100% (5/5 families). This reiterates the fact that rarer diseases manifest in highly consanguineous populations.

Table 8: Syndromic Children (18 patients, 14 families)

Cause	No of children	% of total patients
Branchia-Oto-Renal syndrome	2	1.6%
Pierre Robin Sequence	1	0.8%
Unknown syndrome	5	4.2%
Pendred syndrome	2	1.6%
Waardenburg syndrome	2	1.6%
Walker Warburg Syndrome	1	0.8%
Usher syndrome	1	0.8%
Stickler syndrome	1	0.8%
Peroxisomal disorder	2	1.6%
Metabolic disorder	1	0.8%

4.1.3 Deafness due to environmental causes

Amongst the group with deafness due to acquired, environmental or other causes (22 patients/21 families), the hearing loss was associated with factors due to prematurity in nearly one third of the patients. Overall amongst the total study group prematurity was associated in about 6 percent of all patients. In these patients with prematurity other factors may have contributed to the hearing loss. Two of these patients also had

associated jaundice and one had intracranial haemorrhage. The other causes in this group were cerebral palsy/hypoxia, CMV, otosclerosis, otitis media, meningitis, aminoglycoside toxicity, hypoxia at birth and infective polyneuropathy (Table 9).

Table 9: Acquired/Environmental causes (22 patients/21 families)

Cause	No of children	% of total	% of environmental causes
Hypoxic-ischemia	6	5.1%	27.3%
Prematurity	7	5.9%	31.8%
CMV	1	0.8%	4.5%
?Otosclerosis*	1	0.8%	4.5%
Otitis media**	2	1.7%	9.1%
Meningitis	3	2.5%	13.6%
Aminoglycoside toxicity	1	0.8%	4.5%
Infective polyneuropathy	1	0.8%	4.5%

*This patient had a family history of otosclerosis and ? had cochlear otosclerosis.

**This patient developed sensorineural deafness due to long-standing otitis media

4.1.4 Severity of hearing loss

Severity of deafness was calculated from the most recent audiogram as an average of hearing at frequencies 500, 1000, 2000 and 4000 Hz in the better hearing ear. The severity was classified as mild (20-39 dB), moderate (40-69 dB), severe (70-94 dB) and profound (more than 95 dB). Of the 134 patients, all except 7 patients had symmetrical hearing loss. For the seven patients with asymmetric hearing loss, the severity was classified as for the better hearing ear. As per these criteria nearly 66.6% children had severe or profound deafness. The distribution in this group of Bangladeshi children as a whole was: profound deafness in 53 (39.5%), severe deafness in 35 (26.1%), moderate in 46 (34.3%)(Table 10). Another 8 children had mild deafness and were not included in this study.

Table 10: Severity of hearing loss

Severity of H Loss	Whole group
Profound	39.5%
Severe	26.1%
Moderate	34.3%

4.1.5 Audiometric Shapes

The shape of the audiogram was noted for all the patients and recorded as described in the definitions section (page 12). Taking the whole group, the most common audiogram shape was flat in 77 of the 134 patients (57.4%). The other audiogram shapes were as per table 11.

Table 11: Shapes of audiogram: Total-134 patients

Shape of audiogram	Number of patients	Percent of total
Flat loss	77 (36-profound,19-severe, 22-moderate)	57.4%
Low frequency ascending	12 (1-profound, 6-severe, 5-moderate)	8.9%
Mid frequency loss	11 (2-profound, 5-severe,4-moderate)	8.2%
Gently sloping	25 (14-profound, 4-severe, 7-moderate)	18.6%
Steeply sloping	9 (2-profound, 2-severe, 5-moderate)	6.7%

The subgroup with flat audiogram shape had all levels of severity of hearing loss, though most of the children had severe or profound deafness (36/77). Of the 77 children with flat audiogram shape, 36 had profound deafness, 19 had severe deafness, and 22 had moderate deafness.

In the subgroup with gently sloping audiogram, of the 25 children, 14 had profound deafness, 4 had severe deafness, 7 had moderate deafness. In the low frequency ascending subgroup, of the 12 children, 1 had profound deafness, 6 had severe and 5 had

moderate hearing loss. In the 11 children with mid frequency hearing loss, 2 had profound loss, 5 had severe and 4 had moderate hearing loss. In the 9 children with steeply sloping hearing loss, 2 had profound, 2 severe, 5 moderate hearing loss.

4.1.6 Progression of hearing loss

The information on progression of hearing loss was collected by analysing the serial audiograms in patient records. The hearing loss was classified as progressive if the average pure tone thresholds had worsened by more than or equal to 15dB within a 10 year period.

Progression of deafness was confirmed on audiogram in 14 patients (10.9%) out of 128 on whom this information was available. This number of children with progressive deafness may be an underestimate as for some children the deafness was confirmed at a later age and there were no previous records for these children to decide whether the hearing was stable or progressive.

It is noteworthy that the majority (10/14 [71.4%]) patients with progressive deafness had a genetic cause. The cause was acquired in 1/14 (7.14%) and unknown in 3/14 (21.4%) patients. None of the children with progressive deafness were positive for mutations in *GJB2*. In 13 of these 14 children (92.8%) the deafness had progressed to severe or profound deafness. In 5 of these children the parents were consanguineous.

Table 12: Progressive hearing loss-14 patients

Cause of deafness

Genetic			10
	Non-syndromic		2
		Recessive	2
	Syndromic		6
		Unknown syndrome	1
		Pendred syndrome	1
		Waardenburg syndrome	2
		Peroxisomal disorder	1
		? metabolic disorder	1
	Chromosomal	Trisomy 21	2
Acquired			1
	Otitis media		1
Unknown			3

4.2 Discussion

In this study group of Bangladeshi ethnic origin, the most common cause of hearing loss was genetic in 59.6% patients. Amongst the genetic causes, most of the children had non-syndromic deafness in 57.7% patients. Even after genetic analysis, the cause of deafness remains undetermined in 21.8% patients. As this study population originates from a confined geographic region (Sylhet), there might be some other unknown genes responsible for these cases of deafness of undetermined aetiology.

Mutations in the *GJB2* gene were found to be the cause of deafness in nearly one third of Bangladeshi children with non-syndromic deafness. This has a major implication for the investigation of deaf children of Bangladeshi ethnic origin. If *GJB2* analysis is the first test done for investigation of deafness, it is likely to be positive in one in three Bangladeshi children, obviating the need for further investigation with potential saving of NHS resources.

Amongst the children in this study group with syndromes, most had an undiagnosed (ie undefined) syndrome. In this subgroup of 'unknown' syndrome, all five families reported parental consanguinity. The high level of consanguinity in this group is not surprising since it is well documented that consanguinity reveals rare recessive disorders (Morton 1991).

For children with environmental causes of deafness, perinatal factors were found to be the most common cause of hearing loss in this population. The common perinatal causes were prematurity and perinatal hypoxia.

Interestingly, the proportions of deafness due to genetic, acquired and unknown causes do not differ significantly from proportions described in other populations, which supports the conclusion that deafness due to all causes may be increased in this population of Bangladeshi ethnic origin.

On distributing the whole study group as per their hearing loss, it was found that the patients were quite evenly distributed in all the categories of hearing loss i.e. moderate, severe and profound.

Hearing loss can be classified according to the audiogram shape (Liu & Xu 1994, Parving & Newton 1995). Some generalisations have been made according to the shape of the audiogram. It has been found that autosomal dominant inheritance was strongly associated with sloping audiogram profile (Liu & Xu 1994, Gorlin *et al.* 1995, Martini *et al.* 1996). Usually profound early onset hearing loss has recessive inheritance (Martini *et al.* 1996). Genetic hearing impairment is generally symmetrical. Hearing loss inherited recessively is usually profound and present at an early age, whereas dominant losses are less severe but progressive (Fraser 1976).

In this study group, more than half of the patients had flat shaped audiograms. The next biggest group was patients with sloping audiogram (high frequency loss) in nearly a quarter of patients.

In this study group progression of deafness was confirmed on audiogram in nearly 11% patients. This may be an underestimate as for some of the patients the deafness was confirmed at a later age and there were no previous records for these children to decide whether the hearing was stable or progressive. On analysing these children further, it was found that for more than 70% of these children the underlying cause of deafness was genetic, of which more than half had a syndromic cause of deafness.

5 *GJB2* Testing

5.1 Results

All children with non-syndromic deafness (67 patients/53 families) were seen by the author in the research clinic and consented for testing for mutations in the *GJB2* gene. The Regional Clinical Molecular Genetics Laboratory at Great Ormond Street Hospital and the Institute of Child Health screened for mutations in the coding region, splice site mutations and the 342kb deletion in all the patients recruited for this study. Results are summarised in Table 13.

Of the 67 patients, 20 were confirmed to have biallelic pathogenic mutations in *GJB2* (29.8%)

Table 13: Results of 67 patients (53 families) with non-syndromic deafness

<i>GJB2</i> results	Number of patients	Number of families	Percentage of patients
<i>GJB2</i> positive	20	14	29.8%
<i>GJB2</i> negative	47	39	70.2%

Of these 53 families, 23 families had recessive deafness diagnosed by pedigree analysis alone i.e. either the parents were consanguineous or there were siblings with deafness. Amongst these 23 recessive families, 8 (34.7%) were positive for *GJB2* mutations.

5.1.1 *GJB2* mutations

The mutations and genotypes found in this study group are detailed in Table 14.

Table 14: Mutations in *GJB2*

<u>Mutations</u>	<u>Number of families(patients)</u>	<u>Consanguinity in families</u>
W24X/W24X	6(8)	4/6
IVS1+1/IVS1+1	1(3)	1/1
M1V/M1V	1(2)	1/1
V95M/V95M	1(1)	1/1
W77X/W77X	1(1)	0/1
M1V/V95M	1(1)	0/1
W24X/IVS1+1	2(3)	0/2
Q124X/IVS1+1	1(1)	0/1

Of these 20 probands, 15 (75%) were homozygous for pathogenic mutations and 5 (25%) were compound heterozygote. The parents of these children with mutations in *GJB2* were known to be consanguineous in 7 families (50%). One of the patients not included in the above table was a heterozygote for W24X. It is difficult to say whether she has a missed second mutation or she is a carrier for W24X and her cause of deafness is acquired (as per history available she had ?meningitis in Bangladesh at the age of 3 years). She has been discussed in detail separately in Chapter 6.

5.1.1.1 Severity of hearing loss in the GJB2 positive patients.

The mutation W24X was found to be the most prevalent mutation in this group accounting for 40% of all mutations. An example of a heterozygote for this mutation is shown below (Figure 14).

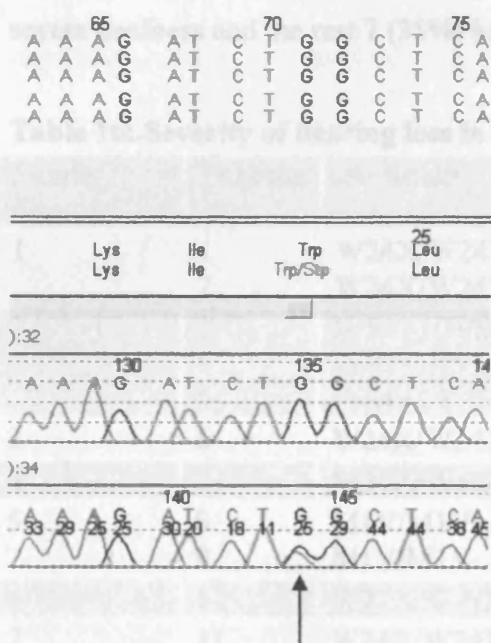


Figure 14: Sequence data for W24X heterozygote, Top trace = normal control, Bottom trace = mutant G>A. Mutation of a G to an A changes the codon for Tryptophan (W) to a stop codon (X).

Polymorphisms in this study are shown in table 15.

Table 15: Polymorphisms seen in the study group (N-Normal sequence)

Mutation of <i>GJB2</i>	Number of patients
R127H/N	10
V153I/N	4
V271/N	3
E114G/N	2
c.15G>A/N	1

5.1.1.1 Severity of hearing loss in the *GJB2* positive patients

The severity of hearing loss in each patient is tabulated in Table 16 below. There are two cases (Family 2 & 9) of discordance of severity between siblings with same genotype. Of the 20 patients from 14 families, 9 patients (45%) had profound deafness, 4 (20%) had severe deafness and the rest 7 (35%) had moderate deafness.

Table 16: Severity of hearing loss in *GJB2* positive patients

Family number	Patients	Mutation	Severity of Hearing loss	Shape of Audiogram
1	1	W24X/W24X	Profound	Flat
	2	W24X/W24X	Profound	Flat
2	3	IVS1+1/IVS1+1	Moderate	Flat
	4	IVS1+1/IVS1+1	Moderate	Gently sloping
	5	IVS1+1/IVS1+1	Profound*	Flat
3	6	W24X/W24X	Moderate*	Flat
4	7	W24X/IVS1+1	Moderate	Flat
5	8	M1V/M1V	Profound	Flat
	9	M1V/M1V	Profound	Flat
6	10	W77X/W77X	Profound	Flat
7	11	W24X/W24X	Severe	Flat
8	12	W24X/W24X	Profound	Flat
9	13	W24X/W24X	Profound	Flat
	14	W24X/W24X	Severe	Flat
10	15	M1V/V95M	Severe	Flat
11	16	V95M/V95M	Profound*	Flat
12	17	W24X/IVS1+1	Moderate	Flat
	18	W24X/IVS1+1	Moderate	Flat
13	19	W24X/W24X	Severe	Flat
14	20	Q124X/IVS1+1	Moderate	Flat

* These patients severity of deafness is in contrast to what is expected of the genotype

5.1.1.2 Carriers of *GJB2* mutations

The heterozygote carrier rates of *GJB2* mutations, W24X and W77X were assessed in the Bangladeshi population. Control samples from anonymized Bangladeshi subjects, collected for a prevalence study on eczema, were used for analysis. These DNA samples were a kind gift from Prof David Kelsell and the analysis was performed by Ms Kerra

Pearce at the Clinical Molecular Genetics Unit at the Institute of Child Health. Carrier rates amongst these unrelated Bangladeshi controls were 2/83 heterozygous for W24X and 1/63 heterozygous for W77X. No homozygotes were detected.

Given the carrier rate for W24X (2/83) in the normal Bangladeshi population, we would expect to find 0.145/1000 children with deafness due to homozygous W24X ($2/83 \times 2/83 \times 1/4$). In this group of 25,809 children we would expect to find 3.7 children with homozygous W24X deafness, if there was no consanguinity. In this study we have found that if we exclude the families with consanguinity, 3 children were W24X homozygous for W24X mutations as expected.

5.1.1.3 Severity of hearing loss and *GJB2* mutations (Genotype/Phenotype correlation)

In order to examine genotype/phenotype correlations, mutations were classified as Inactivating or Non-inactivating (Cryns *et al.* 2004). Mutations which are predicted to severely affect/stop the protein synthesis i.e. W24X, M1V, W77X and Q124X have been classified as Inactivating mutations (I) and those potentially allowing synthesis of some protein i.e. IVS1+1 and V95M have been classified as Non-inactivating mutations (N). In the case of the M1V mutation Methionine is replaced by Valine at position 1. Being the initiating codon, protein synthesis is likely to be severely affected. Figure 15 shows the relationship between mutations in *GJB2* with the severity of hearing loss. Generally it was found that patients who were homozygous for the inactivating mutations (I/I) had severe or profound deafness. Those patients who were compound heterozygote for the mutations (N/I) had moderate hearing loss with the exception of one patient who had severe loss.

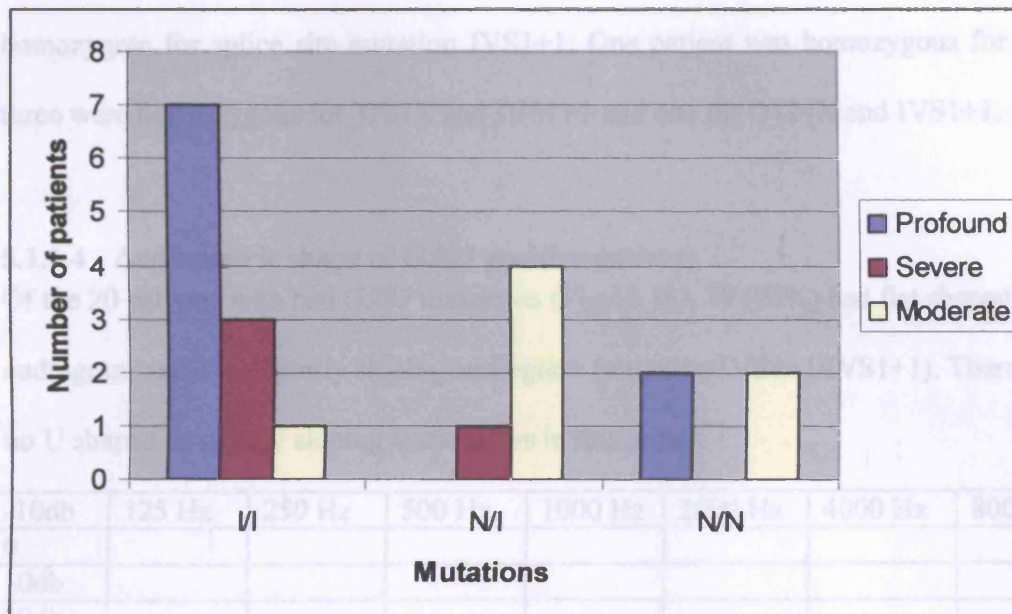


Figure 15: Distribution of severity of hearing loss & *GJB2* mutations

To analyse this further these patients are sub-grouped according to the severity of their hearing loss. Of the 9 patients positive for *GJB2* mutations with profound hearing loss, 7 had protein truncating mutations. Of these 4 were homozygous for W24X and 1 homozygous for W77X, both mutations likely to result in no Connexin 26 protein being synthesized. Another 2 patients were homozygous for the mutation M1V. One patient in this subgroup was homozygous for the splice site mutation IVS1+1, which is assumed to result in reduced protein being formed because of the faulty splicing of the mRNA. One patient was homozygous for the mutation V95M.

Four patients with *GJB2* mutations had severe deafness. Three of them were homozygous for the W24X mutation and one was compound heterozygous for M1V/V95M mutations.

Seven patients with *GJB2* mutations had moderate deafness. Two of the patients were homozygote for splice site mutation IVS1+1. One patient was homozygous for W24X, three were heterozygous for W24X and IVS1+1 and one for Q124X and IVS1+1.

5.1.1.4 Audiometric shape of *GJB2* positive patients

Of the 20 patients who had *GJB2* mutations (Figure 16), 19 (95%) had flat shaped audiogram and 1 had gently sloping audiogram (mutation IVS1+1/IVS1+1). There was no U shaped or steeply sloping audiograms in this group.

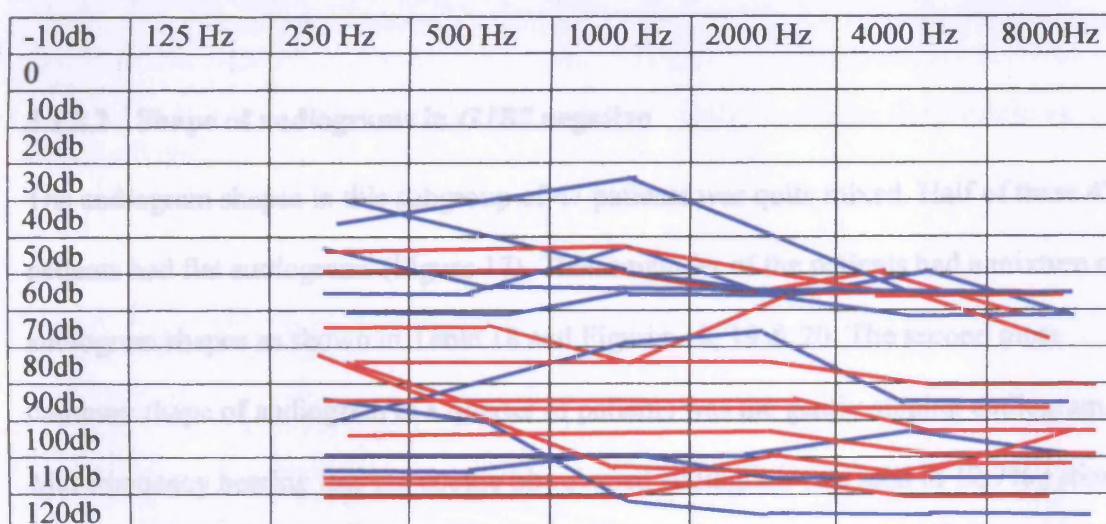


Figure 16: Audiogram shapes in *GJB2* positive patients (Red-Right ear; Blue-Left ear)

5.1.2 *GJB2* negative

Of the 67 patients (53 families) tested for *GJB2* mutations, 47 patients belonging to 39 families were negative for mutations in the *GJB2* gene. All these patients were tested for mutations in exon2, the splice site mutation (IVS1+1) and del342kb.

5.1.2.1 Severity of hearing loss in *GJB2* negative patients

Of these 47 patients in this group, it was found that 19 had profound deafness, 15 severe and 13 had moderate hearing loss. Of these 5/47 had progressive deafness on serial audiograms.

Table 17: Severity of hearing loss in *GJB2* negative patients

Severity of hearing loss	Number of patients	Percentage
Profound deafness	19	40.4%
Severe deafness	15	31.9%
Moderate deafness	13	27.6%

5.1.2.2 Shape of audiograms in *GJB2* negative

The audiogram shapes in this subgroup of 47 patients was quite mixed. Half of these 47 patients had flat audiograms (Figure 17). The remainder of the patients had a mixture of audiogram shapes as shown in Table 18 and Figures 18, 19 & 20. The second most common shape of audiogram in a quarter of patients was the gently sloping audiogram. Mid frequency hearing loss i.e. cookie bite shaped audiogram was seen in 12.7% patients.

Table 18: Shape of audiograms in *GJB2* negative

Shape of audiogram	Number of patients	Percentage
Flat	23	48.9%
Low frequency ascending	5	10.6%
Gently sloping	13	27.6%
Mid frequency loss	6	12.7%

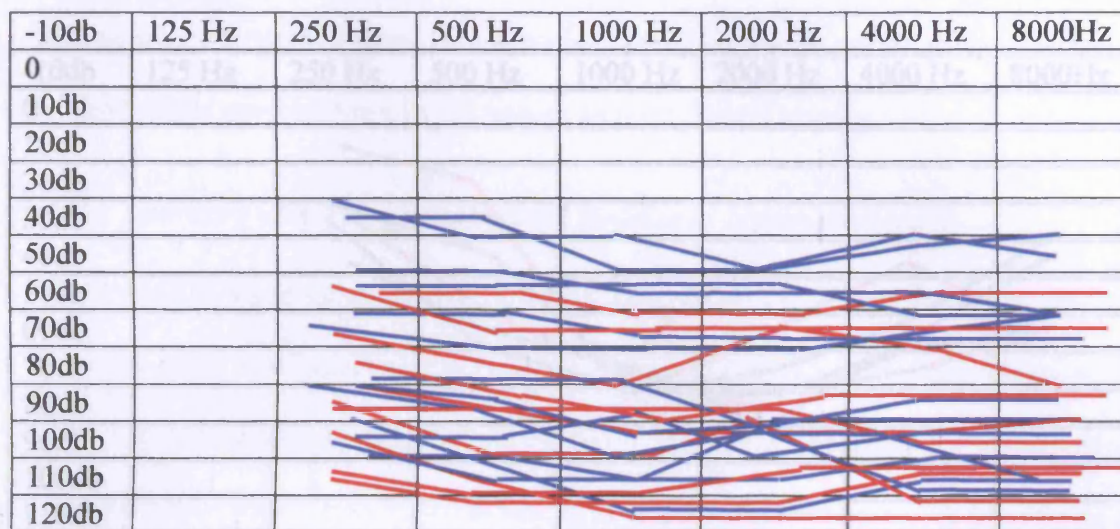


Figure 17: Flat shape Audiograms of Connexin negative patients (Red-Right ear; Blue-Left ear)

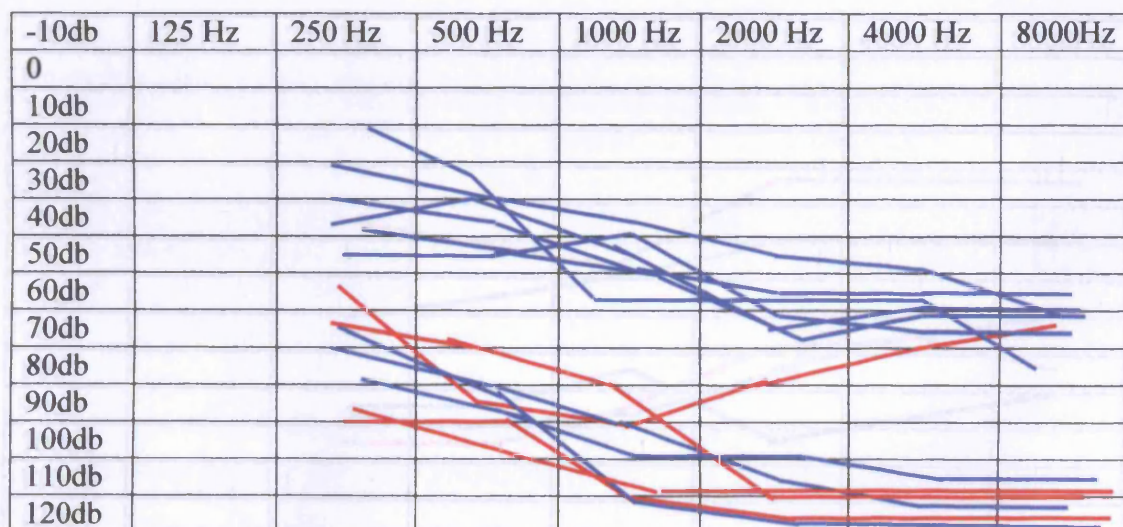


Figure 18: Gently sloping shape audiograms of Connexin negative patients (Red-Right ear; Blue-Left ear)

5.1.3 Comparisons of hearing loss in GJB2 positive & negative patients

The patients who were positive for mutations in the GJB2 gene and those negative for

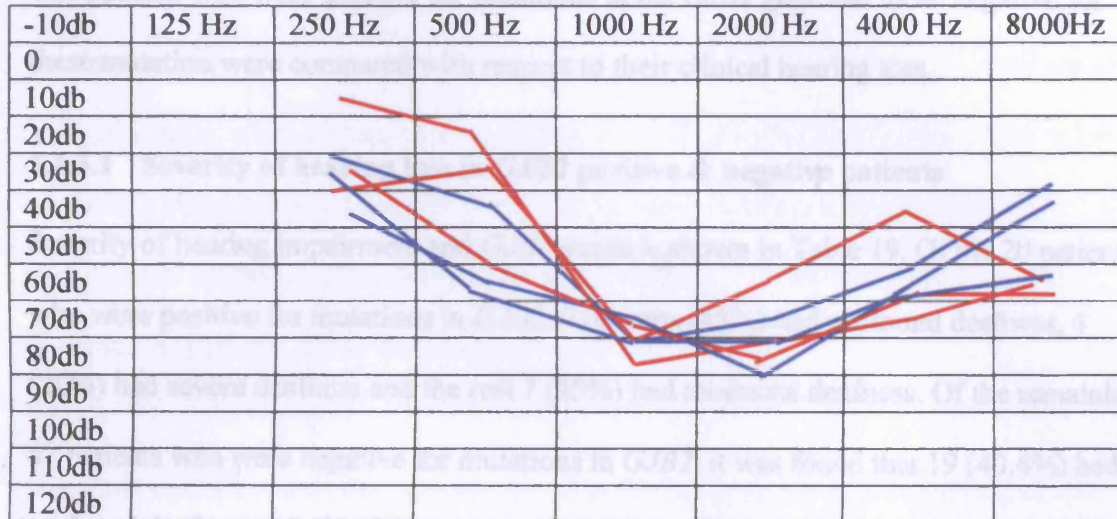


Figure 19: Mid frequency loss shape audiograms of Connexin negative patients (Red-Right ear; Blue-Left ear)

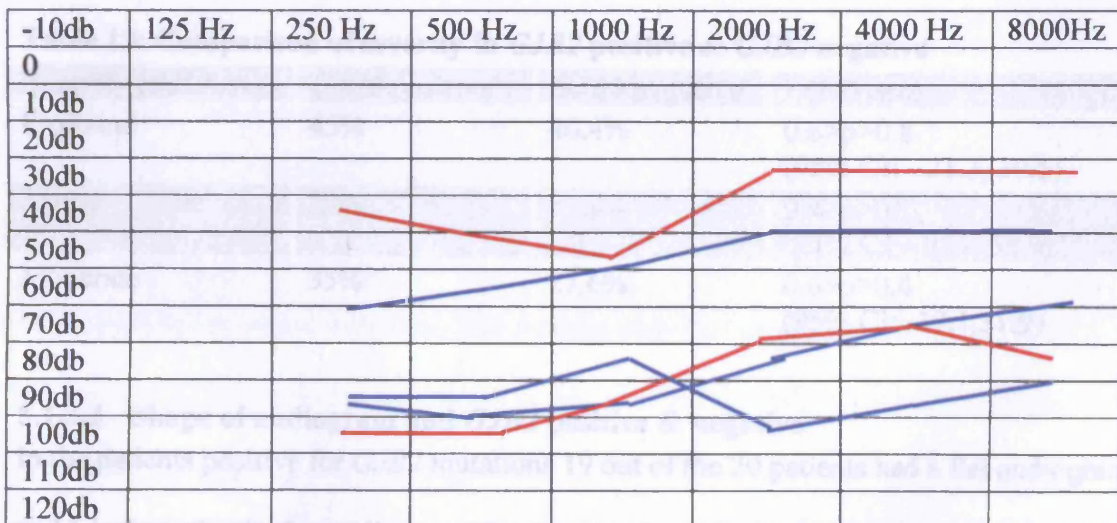


Figure 20: Low frequency ascending shape audiograms of Connexin negative patients (Red-Right ear; Blue-Left ear)

5.1.3 Comparisons of hearing loss in *GJB2* positive & negative patients

The patients who were positive for mutations in the *GJB2* gene and those negative for these mutation were compared with respect to their clinical hearing loss.

5.1.3.1 Severity of hearing loss in *GJB2* positive & negative patients

Severity of hearing impairment and *GJB2* status is shown in Table 19. Of the 20 patients who were positive for mutations in *GJB2*, 9 patients (45%) had profound deafness, 4 (20%) had severe deafness and the rest 7 (35%) had moderate deafness. Of the remaining 47 patients who were negative for mutations in *GJB2*, it was found that 19 (40.4%) had profound deafness, 15 (31.9%) severe and 13 (27.6%) had moderate hearing loss. On comparing these groups with different severity and the *GJB2* mutation status, the difference between them was not significant though the numbers are small.

Table 19: Comparison of severity in *GJB2* positive & *GJB2* negative

Severity of H Loss	<i>GJB2</i> positive	<i>GJB2</i> negative	Difference
Profound	45%	40.4%	0.6>p>0.8 (95% CI: -21.3, 30.5)
Severe	20%	31.9%	0.4>p>0.2 (95% CI: -10.1, 33.9)
Moderate	35%	27.6%	0.6>p>0.4 (95% CI: -17.1,31.9)

5.1.3.2 Shape of audiogram and *GJB2* positive & negative

In the patients positive for *GJB2* mutations 19 out of the 20 patients had a flat audiogram and 1 had gently sloping audiogram. On the other hand in the *GJB2* negative group though nearly half the patients had flat audiogram, the audiogram shapes were quite varied in that group. This is not unexpected as the cause of deafness in the *GJB2* negative could be quite heterogeneous due to a wide variety of unknown mutations.

5.2 Discussion

In this part of the study, in which Bangladeshi patients with non-syndromic deafness of unknown cause were screened for mutations in *GJB2*, 29.8% (20/67) were found to have bi-allelic pathogenic mutations. In other words, nearly every one in every three Bangladeshi children with non-syndromic hearing loss, with environmental causes excluded on history, would test positive for *GJB2*.

Although the most common mutation found in this study group of Bangladeshi ethnic origin was W24X in 55% patients (total-11/20; 8-homozygous, 3-compound heterozygous), there is significant mutational heterogeneity, in spite of the fact that all patients originate from the same Bangladeshi province of Sylhet. The mutations M1V, W77X, V95M, IVS1+1 and Q124X have all been observed more than once in both homozygous and compound heterozygous form, reflecting the fact that the carriers of each are present in the population. We have been able to show a carriage rate of 2 in 83 individuals for W24X and 1 in 63 for W77X. Thus consanguinity will lead to homozygosity for these mutations, and random matings between unrelated carriers, present in this population at significant levels, will lead to compound heterozygosity. In this study the consanguinity amongst these *GJB2* positive families was very high (50%) which is why many families are homozygous for *GJB2* mutations.

The overall carrier frequency in this population approaches the high figures seen in some other populations. The carrier frequency of 167delT in Ashkenazi Jews is approximately

4% (Cohn & Kelley 1999) and a large study of the frequency of the 35delG mutation in 3270 random controls from 17 European countries, estimates a carrier rate of this mutation of 1 in 35 (2.8%) for Southern Europe and 1 in 79 (1.2%) in central and northern Europe.

The mutational spectrum in this British Bangladeshi population is obviously different to the Caucasian British population, a fact which is not just interesting, but which also has diagnostic implications. For example, 6 patients (3-homozygous and 3 compound heterozygous) were positive for the splice site mutation, IVS1+1, which is outside the coding region of the gene. Diagnostic laboratories therefore need to be aware that this is a relatively prevalent mutation in Bangladeshi people and that it needs to be excluded in those with a single coding GJB2 mutation or those who are negative for coding region mutations, since it does not form part of the diagnostic protocol in many laboratories.

GJB2 is now acknowledged to be the commonest cause of genetic deafness worldwide with different ethnic groups having different prevalent mutations. In populations with European ancestry, the 35delG mutation, which causes severe to profound hearing loss accounts for approximately 75% of all mutated alleles (Estivill *et al.* 1998, Gasparini *et al.* 2000, Van Laer *et al.* 2001), (Green *et al.* 1999, Cohn *et al.* 1999, Cryns *et al.* 2004). In non-Caucasians, 35delG is found rarely. In Ashkenazi Jews the 167delT mutation is the most commonly identified mutation (Morell *et al.* 1998), 235delC is most prevalent in Japanese (Abe *et al.* 2000) and R143W is the most common mutation in Ghana (Brobby *et al.* 1998). We found none of these mutations, not even the 35delG mutation, in the

Bangladeshi children. Neither did we find the 342kb deletion upstream of the *GJB2* gene, which is frequently present in patients who only have a single *GJB2* mutation (Lerer *et al.* 2001, Castillo *et al.* 2002) and is the second most frequent mutation causing deafness in the Spanish population (Castillo *et al.* 2002). This deletion disrupts *GJB6* (which is close by and encodes for Connexin 30) but leaves *GJB2* intact but may alter the expression of *GJB2* by deleting an upstream regulatory element.

Previous reports have examined *GJB2* mutations found in families who originate from the Indian subcontinent. Three mutations W24X, W77X and Q124X have been found to be common in individuals from different parts of Indian subcontinent. Rickard *et al.* in their study of 51 multi ethnic deaf patients from London, found 1 patient of Pakistani origin and 1 patient of Bangladeshi origin homozygous for W77X, 1 Indian patient homozygous for Q124X and 2 Indian sibs were heterozygous for W24X (Rickard *et al.* 2001). Scott *et al.* studied six families of which four were Indian in origin (Scott *et al.* 1998). Of these four families mutations in *GJB2* gene were found in three families. One consanguineous family had three individuals homozygous for mutation Q124X; the second family (non-consanguineous) had two individuals homozygous for W24X and the third family (non-consanguineous) had four individuals who were compound heterozygote for W77X and W24X mutations in the *GJB2* gene.

More recently in a larger study, Ghosh *et al.* in their study of 320 consanguineous Indian families with deafness, found the overall incidence of *GJB2* mutations to be 22.5%, which is close to the incidence of 29.8% in Bangladeshi families in this study (Ghosh *et al.* 2004). They also found W24X to be the most common mutation in these families and

also found W77X, Q124X and M1V which are also quite similar to the mutations in our Bangladeshi families. However unlike our study, they found 35delG mutation in 5 families. In another study 35delG was also found in compound heterozygosity with W24X twice among 45 Indian families with non-syndromic deafness along with 4 families who were homozygous for W24X (Maheshwari *et al.* 2003).

Interestingly, the W24X mutation has also been found at high prevalence in populations who are not obviously from the Indian subcontinent but who may have ancestral links there, such as Romany gypsies from Slovenia and Spain, as well as Northern Eastern Hungarians (Alvarez *et al.* 2005).

5.2.1.1 Phenotype in *GJB2*

In this study, patients with *GJB2* mutations presented with all degrees of severity of deafness from moderate to profound (profound-45%, severe-20%, moderate-35%) and this did not differ significantly from those in whom no mutations were found. In other words patients with any degree of hearing loss can have mutations in the *GJB2* gene and thus all children with deafness of unknown aetiology could have *GJB2* associated deafness, and should be offered genetic testing. Similar results have been reported by other authors (Cohn *et al.* 1999). Cohn et al studied 46 patients with *GJB2* mutations 35delG/35delG, 167delT/167delT and compound heterozygotes. The severity of hearing loss in these patients was profound in 65.2%, severe in 23.9% and moderate in 10.8% patients. In addition we also noted significant intrafamilial variability in severity of deafness, especially in the family with the IVS1+1/IVS1+1 mutation where one sibling

had profound deafness and other two had moderate hearing loss. Similar families have also been noted by others (Mueller *et al.* 1999).

The deafness caused by *GJB2* mutations is usually prelingual and congenital, but it is possible that hearing is normal at birth and progresses rapidly during the first few months of life (Green *et al.* 2000). This would mean that although most of the children with *GJB2* deafness will be identified as a part of the newborn screening, some children who have *GJB2* mutations may pass the newborn screening and become deaf during infancy.

In the later years, deafness caused by *GJB2* mutations is usually stable but progression has been reported (Denoyelle *et al.* 1999, Cohn *et al.* 1999). Cohn et al examined serial audiogram for 30 patients with *GJB2* mutations and reported progression of hearing loss in 7 of these patients (4 homozygous for 35delG, 1 homozygous for 167delT and 2 compound heterozygote) (Cohn *et al.* 1999). Two of 16 children with *GJB2* mutations studied by Denoyelle et al over 10 years have showed progression. In our study there were no patients with *GJB2* mutations who showed progression of deafness (Denoyelle *et al.* 1999). Similarly none of the 12 patients in the study by Wilcox et al showed progression (Wilcox *et al.* 2000) and Mueller et al did not report any progression in the serial audiograms of 24 patients with *GJB2* mutations (Mueller *et al.* 1999). Perhaps a reason for this is small sample sizes in some studies (ours included), insufficient follow-up time or data, or difference between the mutations studied in the different publications.

Analysis of the audiograms of the patients in this study showed that most of the patients positive for *GJB2* mutations had flat audiograms while those negative for the mutations

in *GJB2* had quite varied audiogram shapes. This information may be useful clinically in cases where an individual has been found to be a heterozygote for a *GJB2* mutation without a second mutation having been found. In these cases, it is not possible to tell whether the patient happens to be a carrier by chance, a finding which is unconnected with the aetiology of their deafness, or whether they have a second mutation in the gene which cannot be found using available testing methods. If such a patient had an unusual audiogram (i.e. not flat) this may indicate that heterozygosity for *GJB2* is not connected to their hearing loss.

5.2.1.2 Genotype Phenotype Correlation

The correlation between genotypes and phenotypes for *GJB2* mutations has been assessed on a large scale within the last few years.

Cryns et al reported results of a multicentre study from Europe and United States involving 277 patients. They suggested that inactivating mutations, which include stop or frameshift mutations show significantly more severe hearing loss than those caused by non-inactivating mutations i.e. missense mutations (Cryns *et al.* 2004). We found a similar relation in our small group of patients. Most of the patients (8/9-88.9%) who were homozygous for protein truncating mutations i.e. W24X, W77X were found to have severe or profound hearing loss. Only one patient out of eight, homozygous for W24X had moderate deafness, although one patient homozygous for V95M had profound deafness, which is contrary to this theory. Two patients homozygous for the mutation M1V at the initiating codon, were also found to have profound deafness, which is perhaps not surprising, since although this is a 'missense' change it is unlikely that any protein,

Connexin 26, could be synthesized when the initiating codon is mutated. Similarly two patients homozygous for the splice site mutation, IVS1+1, had moderate deafness, which may be because splice mutations may often allow production of some normal mRNA thereby permitting some protein synthesis to occur. All patients except one (4/5-80%) with compound heterozygous mutations had moderate deafness (3-W24X/IVS1+1; 1-Q124/IVS1+1) and one had severe deafness (M1V/V95M).

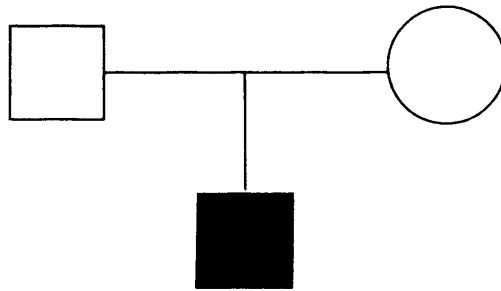
Another large multicentre study involving 1,531 patients from 16 countries by Snoeckx *et al* analysed *GJB2* genotype and phenotype (audiometry) in patients with non-syndromic autosomal recessive hearing loss (Snoeckx *et al.* 2005). They identified 83 mutations in the study group and classified 47 as non-truncating mutations and 36 as truncating mutations. They reported that the degree of hearing loss associated with biallelic truncating mutations was significantly more severe than the hearing loss associated with biallelic non-truncating mutations such as M34T, V37I and L90P where hearing loss was mild to moderate deafness. On the other hand 35delG/R143W and 35delG/delG*GJB6* had significantly more severe deafness than 35delG/35delG.

From these studies it can be inferred that phenotypes caused by truncating *GJB2* mutations are more severe than those caused by missense mutations. Thus genotype is a fundamental factor in predicting phenotype. However, phenotype variations among the same genotypes exist, both within and between families. These variations may be due to factors such as alterations in promoter regions and additional genes (del Castillo *et al.* 2002), modifier genes (Abe *et al.* 2001) and environmental factors.

Such correlations may help in predicting the mutation as per the severity of hearing loss i.e. someone with a profound hearing loss seen in the clinic can be assumed to be homozygous for an inactivating mutation. The clinician still has to wait for the final genetic analysis. These correlations may be also useful in predicting the likely course of hearing loss and may help in making decisions regarding management of the patient.

6 Case Studies

6.1 Case study number 44



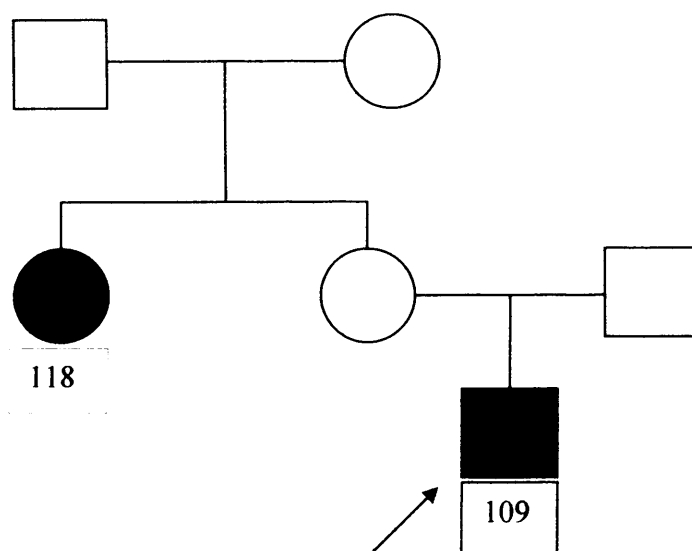
Case number 44 is 14 years old at present. He developed *Haemophilus meningitis* at the age of 1 year, during which his parents suspected hearing loss. He was confirmed to be profoundly deaf and hearing aids were given. Over the last 8 years his deafness has progressed. He communicates by speech. There was nothing significant in the prenatal or perinatal history and developmentally he was normal. His parents are not consanguineous. There is no family history of deafness. His general physical and ENT examination were normal. On investigating, it was found that all his blood tests were normal. CT scan of temporal bones showed bilateral mildly dilated vestibular aqueducts and normal cochleae. Perchlorate scan appeared to show 22% discharge although the baseline was highly variable due to movement artefact. At this stage a diagnosis of Pendred syndrome was considered and he was referred for a second opinion to a professor of neuroendocrinology. In view of subtle dilated vestibular aqueducts, MRI was

requested and also genetic analysis for Pendred mutations (the *SLC26A4* gene) was requested.

The consensus opinion was that in view of the fact that there was a clear association between the apparent onset of hearing loss and the episode of meningitis, the more likely cause of deafness was meningitis. It was thought that the perchlorate discharge test may have been artefactual, while the vestibular aqueducts only showed minor changes. The MRI was reported to show normal endolymphatic sacs. In addition his Pendrin level 1, 2 and 3 mutation analyses were negative. In view of these, the cause of his hearing loss was thought to be due to meningitis.

The interesting feature of this case was the difficulty in reaching a diagnosis/cause for the hearing loss. It is interesting how the diagnosis changes as the results of complex investigations unfold. The results of initial investigation pointed towards the diagnosis of Pendred syndrome, which became less likely as other results came through. After various expert opinions the cause of deafness was thought to be meningitis.

6.2 Case studies number 109 & 118



Case number 109 is nephew of case number 118 (her sister's son). Case 109 is 5 years old at present. His parents suspected hearing loss when he was 9 months of age and was confirmed to have severe hearing impairment at 10 months of age, soon after which he was given bilateral hearing aids. He communicates mainly by British Sign Language. There was nothing significant in the prenatal or perinatal history and developmentally he was normal. His parents are not consanguineous and he is their only child. There was a family history of deafness i.e. his maternal aunt (Case 118). She was believed to have become deaf after a possible episode of meningitis in Bangladesh. His general physical and ENT examination were normal. On investigation, it was found that all his blood tests (Full blood count, urea & electrolytes, liver function tests, thyroid function tests) were

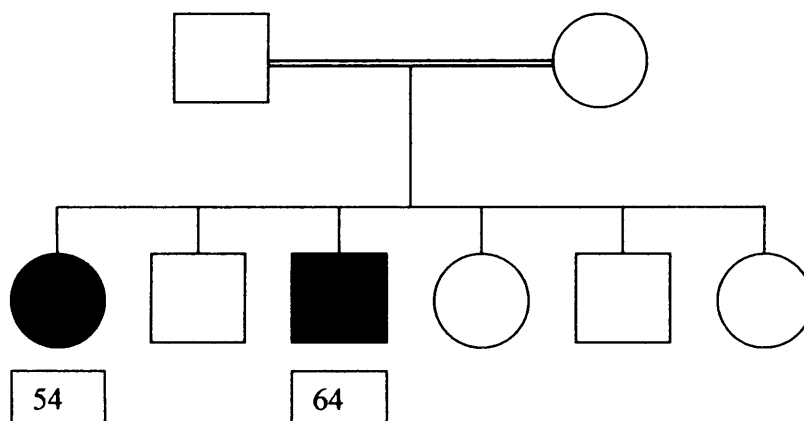
normal. His CT scan, renal ultrasound and ECG were normal. On genetic analysis he was found to be homozygous for W24X mutations in the *GJB2* gene.

His maternal aunt Case number 118 is 24 years old at present. Her hearing loss was confirmed at the age of 9 years. Parents associate the onset of hearing loss with an episode of fever with fits at the age of 4 years (? meningitis). The delay in diagnosis of her hearing loss was because she was living in Bangladesh before 9 years of age. MK communicates by speech and has very well developed language. She has profound hearing loss and her hearing loss has been stable over past 15 years. No significant factors were elucidated in her prenatal or perinatal history. Her parents are not consanguineous. She has four siblings, all of whom have normal hearing. The only family history of hearing loss is her nephew (case 109). Her general physical and ENT examination were unremarkable except for a left preauricular tag. All investigations were normal for her.

Her genetic testing revealed that she was heterozygous for the W24X mutation in *GJB2*. No second mutation to account for her hearing loss in *GJB2* was found. This heterozygous status for W24X mutation could suggest that either she has another mutation in *GJB2* which cannot be detected at present, or she is a carrier for W24X and the cause of her hearing loss is due to the illness in childhood. She is also negative for the A1555G mutation.

This family is interesting as the nephew has prelingual profound hearing loss which is clearly due to homozygous W24X mutations. He mainly uses sign language with limited speech. It was assumed that his aunt's deafness would also be genetic due to the same cause. However, delving further into her history together with investigations and the fact that she was postlingually deaf, suggested an environmental cause of deafness, supported by the finding that she was clearly a heterozygote for W24X. She is likely to be a carrier by chance.

6.3 Case study number 54 & 64



Cases number 54 and 64 are siblings with hearing loss. Case 54 is at present 19 years old. She was suspected to have hearing loss at the age of 4 years. This was confirmed and she was subsequently given hearing aids. She has profound hearing loss which has been stable. She communicates by British Sign Language. There was nothing significant in her prenatal or perinatal history. Her parents are consanguineous. She has six siblings, of whom five have normal hearing and one has hearing loss (case 64). Her general physical and ENT examination were unremarkable. All investigations were normal except for her genetic testing which revealed that she was homozygous for the V95M mutation in *GJB2*.

Case 64 is at present 14 years old. His hearing was checked at 2 months of age because of his sister's hearing loss. He was confirmed to have hearing loss at 7 months of age and was subsequently fitted with hearing aids. He also has profound hearing loss which has been stable. He also communicates by British Sign Language. There was nothing significant in his prenatal or perinatal history. His general physical and ENT examination were unremarkable. All investigations were normal in him. His genetic testing did not reveal any mutations for *GJB2*, in particular he does not have the V95M mutation on either allele.

Siblings with similar hearing loss born to consanguineous parents are expected to have the same mutations in the *GJB2* gene. It is of interest that one of them is homozygous for the mutation and yet the other sibling does not show any evidence of the mutation. One possible explanation could be that there are two recessive genes causing deafness in this family. The V95M mutation in *GJB2* could be one of them and we are not able to detect the other at present.

7 General results

7.1.1 Languages used at home

Of the 53 families seen in the research clinic, in 11 families (20.8%) the parents could not read English or Bengali and could communicate only verbally in Sylheti. These 11 families were given audio tapes in Sylheti as further information about the research. The parents in the other 42 families could read either English or Bengali and were given information leaflets in the language they preferred.

In all the 118 children on whom the information was available, Sylheti was spoken at home. For 49 children the only language used at home was Sylheti. In families of 39 children English as well as Sylheti was spoken at home. Sign language was being used at home for 30 children in addition to Sylheti or English.

Table 20: Languages used at home

Languages used at home	Number of patients	Percentage
Sylheti only	49	41.9%
Sylheti & English	39	33.1%
Sylheti & Sign language	23	19.5%
Sylheti & English & Sign language	7	5.9%

Information on child's first/preferred language was available for 118 patients (Table 21).

It was found that for 30 children the first language was English and Sylheti in 41 patients. A significant number (47; 40.3%) used British Sign Language as their first

language. As expected, children's preferences of language do differ from those of their parents.

Table 21: Child's first language

First Language	Number of patients	Percentage
Sylhetti	41	34.7%
English	30	25.4%
British Sign language	47	39.8%

7.1.2 Mode of communication

Of the 120 children on whom this information was available, 59 communicated using speech, 16 communicated using gestures, 32 were exclusively British Sign language users and 13 children used a combination of speech and sign language.

Table 22: Mode of communication

Communication	Numbers	Percentage	Severity of deafness
Speech alone	59	49.2%	Profound-4, Severe-21, Moderate-34
Speech & Sign both	13	10.8%	Profound-6, Severe-5, Moderate-2
Sign language only	32	26.7%	Profound-32
Gestures	16	13.3%	Profound-9, Severe-3, Moderate-4

Of these 48 sign language or gesture users, in 30 children's families (62.5% of sign users) sign language was also being used at home by the parents to communicate with the child. Most of these parents who learnt sign language were young parents who were very keen to be involved in their child's overall development and education.

7.1.3 Initial suspicion of Hearing loss

Of the 119 patients, in the majority of the patients the hearing loss was first suspected by parents (59/119). In 34/119 hearing loss was suspected by child development team, 13/119 by school teachers, 9/119 by health visitors and 4 of these were picked up on the

newborn hearing screening programme (Date of birth's of these children were 4/7/03, 27/10/03, 2/3/04, 15/5/04). In 15 patients this information was not available. Amongst this study group the neonatal hearing screening had been performed in only three patients but it is expected that in future most of the children with hearing loss will be detected at the neonatal hearing screening.

Table 23: Initial suspicion of hearing loss

Hearing loss- initial suspicion	Number of patients	Percentage
Parents	59	49.6%
Child development team	34	28.5%
School teachers	13	10.9%
Health visitor	9	7.5%
Newborn hearing screening	4	3.4%

7.1.4 Age of confirmation of hearing loss

The average age of confirmation of hearing loss in this group of patients as a whole was at the age of 3 years 5 months (range-1 month to 15 year). On dividing these children as per the decade in which they were born, it is quite apparent that with the passage of time (Table 24) the age of detection of deafness has fallen in this study group. This indicates that the audiology services have improved over the decades.

Table 24: Age of confirmation of hearing loss in different decades

Children Born in decade	Mean age of confirmation of Hearing Loss	Mode age of confirmation of hearing loss
1980-1989	4 years 5 months	3 years
1990-1999	3 years 1 month	2 years
2000 onwards	1 year 3 months	Less than 6 mths

7.1.5 Time between confirmation of diagnosis and hearing aids

This data was collected as per the patient records. The duration between the confirmation of hearing loss and patient actually receiving the hearing aids was recorded. This duration ranged from 2 weeks to 14 months. The average duration for 97 patients for whom this data was available was 8 weeks. Of these 97 patients, 75 patients (77.3%) received their hearing aids within 6 weeks and 51 (52.5%) within 4 weeks. In most of the other patients the delay in hearing aids was due to the parents not being keen for the child to wear hearing aids due to social reasons.

Table 27: Duration between diagnosis and hearing aid

Time between diagnosis & hearing aid	Number of patients	Percentage
Less than 4 weeks	51	52.5%
Less than 6 weeks	75	77.3%
More than 6 weeks	22	22.7%

7.1.6 Attitudes in this study group

7.1.6.1 Reason for participation in this research

Families who were seen in the research clinic were asked their reasons to participate in this research project. Of the 55 patients, 15 participated as they were interested to find the cause of their/their child's hearing loss. Another 18 participated for the sake of their/their child's future and the rest 22 participated both to find out the cause of deafness and for the sake of their own/their child's future.

Table 28: Reason for participating in research: Total-55

Reason for participating in research	Number of patients	Percentage
To know cause of hearing loss	15	27.7%
For the sake of child's future	18	32.7%
Both to know cause & child's future	22	40.0%

7.1.6.2 Completing the family

These 55 parents were also asked if a child with hearing impairment affected their decision about having other children. Of these 51 (92.7%) replied that a deaf child in the family did not affect their completing the family. Two parents replied yes and another 2 did not comment. This was also evident from the fact that the hearing impaired child was not the youngest child in the family.

Table 29: Completing the family: Total-55

Effect of deaf child on having more children	Number of families	Percentage
No effect	51	92.7%
Effecting completing family	2	3.6%
No comments	2	3.6%

7.1.6.3 Genetic Counselling

All these 55 patients/parents were asked if they would be interested in genetic counselling for planning their family or their child's family in the future. Of these 39 (70.9%) said yes to genetic counselling. Of the rest 4 (7.2%) were not sure, 6 (10.9%) answered that they were not planning any more children (did not want to comment) and 6 (10.9%) said no to genetic counselling. Many of these parents went on to have further children maybe because they were not aware of the genetic factors. It may be that these parents wanted genetic counselling so as to understand the reasons for their child's deafness.

Table 30: Views on genetic counselling: Total-55

Genetic counselling	Number of patients	Percentage
Interested	39	70.9%
Not interested	6	10.9%
Not sure	4	7.2%
No comments	6	10.9%

7.1.6.4 Views on prenatal testing

The parents/patients were asked their views on prenatal testing to find out if the child could have hearing loss. They were explained in brief that chances of miscarriage during the testing were around 2%. More than half of the families (33/55, 60%) clearly answered that they were not interested in prenatal testing. 11/55 (20%) said they would be interested in prenatal testing if offered. 11/55 (20%) were not sure of their answer and said they did not know what they would do in that situation.

Table 31: Views on prenatal testing: Total-55

Prenatal testing	Number of patients	Percentage
Not interested	33	60.0%
Interested	11	20.0%
Not sure	11	20.0%

Of the 11 patients/parents who showed interest in prenatal testing, because of the sensitivity of the issue, only three were asked if they would continue with the pregnancy if the child was found to be carrying deafness causing mutation. Of these only 1 answered that they would not continue with the pregnancy and the rest were not sure. Rest of the 9 were not asked the question about continuing pregnancy, because of the sensitivity of the issue in this population and was advised by the liaison worker not to ask the question to these parents.

7.2 Discussion

7.2.1.1 Communication in this population

All the Bangladeshi families recruited for this study originate from Sylhet and Sylhetti is spoken in all the households as a preferred language at least by the parents. The Sylhetti

language does not have a written script. Awareness of this before the project was started meant the information about the research was prepared as leaflets in English, Bengali and as audio tapes in Sylheti. In this study it was found that parents could not read English or Bengali in one fifth of the families and they were given audio tapes in Sylheti as information about research. This has health care and social implications far beyond this project as this may be the reason a large number of these families do not attend clinic appointments, leading to poor healthcare.

Sylheti was spoken by all the families seen in this study. In the majority of families (~42%) this was the only language spoken at home and these children with deafness would have to manage Sylheti at home and learn English in school. For the rest of the families some other language was being used at home i.e. English or British sign language.

Another interesting finding was the child's preferred or first language. It was not surprising that for only one third children (34.7%) the first language was Sylheti. This can be explained by the fact that most of these children were the second generation in UK. For the remaining two thirds the first language was English (25.4%) or British sign language (39.8%).

The language barriers in this Bangladeshi community could be contributing to the high unemployment in this population. It has been reported that hearing impaired children from the ethnic minority communities had worse educational achievements as compared to their Caucasian peers (Cohne *et al.* 1990). It has also been reported that these children,

for whom English is not the home language, are more socially isolated (Cohne *et al.* 1990). Another study reports that poor educational outcome and social isolation may be due to late diagnosis and inadequate rehabilitation (Webb 1996).

Children included in this study communicated using both verbal and non-verbal modes of communication. For the majority of children (approximately 40%) the only mode of communication was signing (British sign language or gestures if young). Of these 92% children had severe to profound deafness. Nearly 10% of children communicate using both speech and sign language. Of these about 85% children had severe to profound deafness. This seems to be the group of children who were aided and developed speech and have learnt both modes of communication and use either according to the need. The remaining 50% children use speech to communicate. Most of these children (~75%) have moderate to severe deafness. This has an implication on integration of these children into the wider community as those using only Sign language may feel isolated.

It was interesting to find out that amongst the children using signing at home; use of sign language was quite acceptable to most of the parents (~71%). This is quite commendable as anecdotally parents in this community do not like their children to wear hearing aids in public because of the social stigma attached to it. Another interesting finding was that a majority of the parents (~60%) of these children using sign language were actually using sign language to communicate with them at home. This is a reflection of the commitment of these parents to the children as they would have made special efforts to learn British sign language. Most of the parents in this study group were normal hearing.

7.2.1.2 Diagnosis of Hearing loss

It is not surprising that in this study in the majority (50%) of the patients, the hearing loss was first suspected by the parents. The next biggest group was the initial suspicion of deafness by the child development team in nearly 30% children. In others the hearing loss was first picked up by the school teachers or the health visitors.

The Joint Committee on Infant Hearing endorsed Universal Newborn Hearing Screening in 2000 (Guilford *et al.* 2000). The aim was to provide hearing screening to all newborns before the age of 1 month, with confirmation of hearing loss in infants who do not pass the initial or a subsequent screening by the age of 3 months. Definitive treatment can then be started before the age of 6 months (Baroch 2003). The two main methods used in the screening are otoacoustic emission and automated auditory brainstem response. The limitation of the screening programme is that not all cases of childhood hearing loss will be detected. The neonatal screening may not identify children with progressive hearing loss, which is approximately 15% of preschool children with sensorineural hearing loss (Hone & Smith 2002). The main aim of the screening is to diagnose and manage early so that a child can develop normal language.

Before the early hearing detection programmes were implemented, the average age of detection of hearing loss was 1.5 to 3 years which is beyond the beginning of the critical interval for speech and language acquisition (Schrijver 2004). Delay in the diagnosis of hearing loss has an impact on development of language and communication skills and

psychosocial development (Yoshinaga-Itano *et al.* 1998). It can also lead to isolation later in life (Baroch 2003).

The newborn hearing screening programme was started in these boroughs in 2000. In this study group four children were suspected to have hearing loss during the newborn screening. It is expected that in future more children will be picked up by the newborn screening. With the introduction of the newborn screening programme the average age of detection of deafness will go down substantially and will be helpful for these children in the long run.

The average age of confirmation of hearing loss in this study group was 3 years 5 months. To analyse it further, the study group was divided into children born in 1980s, 1990s and after 2000. It was observed that the age of confirmation has dropped down from 3 years in 1980s to 2 years in 1990s and further down to less than 6 months in children born after 2000. This could be attributed to increased awareness on the part of parents or schools and improvement in health service provision.

Deafness should be diagnosed at an early age as early rehabilitation is important to provide better quality of life. Efforts have to be made to increase awareness amongst physicians and general public. Clear guidelines have to be established for evaluating children with deafness.

Providing support to families through educational services, audiology services and social services help. It has been shown that earlier the child and the family get this support, more the benefit (Yoshinaga-Itano *et al.* 1998). The language development can be improved through the use of language support programmes and any residual hearing can be used through adequate amplification.

The time interval between the confirmation of hearing loss and the patient actually getting the hearing aid was recorded from the notes. Once the hearing loss has been diagnosed, the child should be rehabilitated as soon as possible in interest of child's overall development. From the data collected in this study it was found that more than half of the children were aided within 4 weeks of confirmation of their hearing loss and nearly 78% within 6 weeks. Most of the patients who received the hearing aids later than this were because the parents were not initially keen for the children to use hearing aids.

7.2.1.3 Attitudes about hearing loss & genetic testing

The attitudes to genetic testing vary amongst the hearing and deaf patients (Middleton *et al.* 1998). Those patients seen in the clinic for this study were asked a few questions to assess their views about the research and genetic testing. It goes without saying that those parents who agreed to participate in the research were keen to find out the cause of their child's hearing loss. All those who participated in the research were either just curious to know the cause of their child's hearing loss or wanted to know the cause for the sake of their child's future. Of course those parents who were not interested in the aetiology, declined to participate in the research.

On looking at the family tree and asking the families it was quite clear that in more than 90% of the families, a deaf child in the family did not effect their decision about having further children. On the other hand 4% patients did respond by saying that they felt apprehensive about having any further children after the birth of a deaf child. Another 4% did not want to comment.

Middleton et al found a predominantly negative view to genetic testing amongst delegates at the Deaf Nation conference in UK (Middleton *et al.* 1998). More than 50% of these delegates thought that genetic testing would do more harm than good. Another large survey from UK (644 deaf, 143 hard of hearing and 527 hearing subjects) revealed that culturally Deaf participants were significantly more likely to say that they would not be interested in prenatal testing for deafness (Middleton *et al.* 2001). Of the hearing subjects who would consider prenatal testing, 62-91% subjects would use this information to prepare themselves for the needs of the child. Only a small minority said they would have prenatal diagnosis to terminate the foetus.

Another study from US of hearing parents (96) of deaf children showed that most of the parents had a positive attitude towards genetic testing for deafness including prenatal testing, but none of them would use the information to terminate the pregnancy (Brunger *et al.* 2000a).

Another study of a mixed group of 337 subjects from US revealed that there was difference in the attitudes towards prenatal testing for hearing loss between culturally

Deaf subjects and persons with hearing loss who are culturally associated with hearing community (Stern *et al.* 2002). Culturally Deaf subjects in this study felt that genetic testing for deafness will have negative effect on the Deaf community. Most of the culturally Deaf subjects were not keen on prenatal diagnosis for hearing loss as compared to those culturally associated with hearing community.

More recently general attitudes of hearing, hard of hearing and deaf population towards genetic testing for hearing loss were examined. It was found that 85% of hearing and 62% of congenitally deaf or hard of hearing individuals would allow genetic testing for their child (Martinez *et al.* 2003). This indicates increasing acceptance of genetic testing for hearing loss.

7.2.1.4 Genetic Counselling

As a part of looking at the attitudes of parents in this study group, the parents were asked if they would be interested in coming to a genetic clinic for genetic counselling. More than 70% patients wanted to come for genetic counselling if invited. About 11% were not interested and 11% did not want to comment at that stage. The remaining 7% were not sure at that time.

Parker *et al* have reported about a survey on patients expectations from genetic services (Parker *et al.* 2000). Their study revealed that a sizeable number of families with deaf children were not being offered genetic services. Those families who had genetic counselling had positive and negative experiences in equal proportion. The families were primarily interested in the cause and risk of recurrence. A large number of those who

were not offered genetic counselling would have liked to attend counselling. Also a large proportion of families who have had genetic counselling could not remember what recurrence risks were quoted. This could be minimised by summarising the session in a letter to the family and offering a follow up appointment.

7.2.1.5 Prenatal Diagnosis

Further on to the questioning about the attitudes, these parents were asked about their views on prenatal testing. Nearly 60% patients were quite clear that they were not interested in prenatal testing or diagnosis. Some of these themselves mentioned that they were not going to do anything about it, so there was no reasoning to unnecessarily take the risk of miscarriage. Another 20% said that they would be interested in the prenatal testing for deafness if offered and the rest 20% were not sure. In summary the majority of parents in this population of Bangladeshi ethnic origin were not keen on prenatal diagnosis.

The attitudes on prenatal testing of this Bangladeshi population in this study are quite in contrast to the study by Middleton on the same subject (Middleton *et al.* 2001). Prenatal diagnosis would be considered by 49% of hearing parents who have a deaf child and by 21% of deaf parents. Within these groups, parents considering termination of pregnancy is a very small minority (Middleton *et al.* 2001).

Brunger *et al* surveyed normal hearing parents of deaf children in USA (Brunger *et al.* 2000b). It was found that 96% parents had positive attitude towards genetic testing. Of

these parents 87% were interested in prenatal testing for deafness. This is very different from our results and could be a reflection of ethnic background of parents in our study.

Another study was carried out by Ryan et al on 104 pregnant women attending Aberdeen maternity hospital (Ryan *et al.* 2003). They reported that 72% of the study group would want to know if they were carrier of a gene causing deafness and 74% would be interested in prenatal testing for deafness. Though amongst these only 7% would terminate an affected pregnancy.

8 Discussion

This study of prevalence and causes of deafness in children of Bangladeshi origin resident in East London up to 16 years of age demonstrates at least 3.96 per 1000 are deaf, which is 2.4 times the national average of 1.65/1000. In the British Bangladeshi population our analysis suggests that consanguinity contributes to the raised prevalence of deafness. However removal of those children born of consanguineous marriages from the calculation does not reduce the prevalence to the national figure and so it is likely therefore that environmental factors may also play a part in the high prevalence of deafness. Interestingly, the proportions of deafness due to genetic, acquired and unknown causes do not differ significantly from proportions described in other populations, which supports the conclusion that deafness due to all causes may be increased in this population.

Testing for mutations in the *GJB2* gene in children with deafness, in whom environmental causes have been excluded by history, also reveals important information. Mutations in *GJB2* have been shown to account for 34.6% of recessive deafness in the Bangladeshi population but the mutational spectrum is markedly different from that seen in the UK Caucasian population. As 1 in 3 non-syndromic hearing impaired Bangladeshi child is expected to have mutations in *GJB2*, all children with deafness of unknown cause should offered *GJB2* testing regardless of the severity of hearing loss. High consanguinity

and a high carrier rate for *GJB2* mutations in Bangladeshi population also indicate that *GJB2* mutations are common in this population.

In European populations the most common mutation in this gene is 35delG, which accounts for 70% of mutant alleles. Among Bangladeshi's we did not find a single case of this mutation, but the commonest mutation was W24X, followed by IVS1+1, M1V, V95M and W77X. This observation highlights the importance of documenting ethnicity when genetic testing is requested. Although East London is home to the highest concentration of Bangladeshi children there are many other regions in the UK where smaller numbers of Bangladeshi families have settled, and genetic laboratories may need to adapt their standard testing protocols in order to detect some of the common Bangladeshi mutations.

Most of the children with *GJB2* mutations have hearing loss in the severe to profound category, a reflection of the commonest mutation, W24X, which is predicted to inactivate the gene. However the occurrence of the non-coding splice mutation IVS1+1, and the missense mutation V95M, associated with a milder hearing loss (Cryns *et al.* 2004), means that phenotype of hearing loss in this population cannot be used as a predictor of *GJB2* mutation status. The children with *GJB2* mutations in this study had a non-progressive hearing loss. The shape of the audiogram is an important finding. Non-flat shape of the audiogram can suggest that the *GJB2* may not be the cause of deafness in that patient. Consanguinity is associated with rare syndromes and syndromic patients tend

to have progressive hearing loss. It is important to keep these children on long term follow up for audiological assessments.

With the implementation of Newborn Hearing Screening the average age of diagnosis of deafness from all causes is expected to reduce from 3 years 3 months in this study to less than 6 months. Given the demographics of this population, with a high proportion of young adults and a high fertility rate, we suggest that *GJB2* testing is offered to all parents of deaf children very soon after diagnosis of deafness. Many mothers become pregnant again whilst aetiological investigations are underway and genetic information will not only enable parents to make decisions about family planning but may help to guide health professionals in the management of the deaf child. In this study it was found that having a deaf child, does not appear to affect the decision of the families to have more children. So may be genetic counselling and testing will not make much difference to this community although parents may still want genetic counselling.

Finally, there remain significant numbers of children in whom the cause of deafness either remains unknown (usually singleton cases in their families) or the diagnosis is autosomal recessive deafness with no mutation in *GJB2*. There are 39 such families in our study, 29 of which have non-consanguineous parents. For rest of the 10 families with consanguineous parents, linkage mapping will be carried out in future to identify other genes.

This suggests that there remain other genes responsible for childhood deafness among Bangladeshi's and that they may be present at a significant frequency in this population.

The population in Tower Hamlets is exceptional in contrast to regional and national trends. The majority of population is very young and expanding due to high fertility rate. This community has large families and a high proportion of genetic hearing loss. This suggests that genetic services are useful in this population. In dealing with this community it is important to be culturally sensitive and using the right language. The local services in the future should be planned keeping all this in view.

9 Recommendations

- Connexin 26 testing should be done before requesting other aetiological investigations in non-syndromic deafness in children of Bangladeshi ethnic origin as mutations in *GJB2* are the cause of deafness in nearly one in three British Bangladeshi children with non-syndromic deafness.
- The spectrum of mutations in *GJB2* is completely different to the Caucasian population which should be taken into account when genetic testing is requested.
- Genetic testing should be offered early to this population where consanguinity is common and birth rates are high, in order to provide genetic counselling for parents and to assist medical management.
- Since the prevalence of severe to profound congenital sensorineural hearing loss seems unusually high in this population, Tower Hamlets PCT and Hackney PCT would need to take this into account in their budgeting for cochlear implantation in their paediatric population

10 Appendixes

10.1 Publications from this research

Bajaj Y, Sirimanna T, Quader P, Jenkins L, Loughlin S, Pearce K, Cortina-Borja M, Albert D, Bitner-Glindzicz M (2006). Aetiology of sensorineural hearing loss in British Bangladeshi children in East London. In preparation for submission to Clinical Otolaryngology.

10.2 Presentations from this research

Bajaj Y, Sirimanna T, Albert DM, Qadir P, Jenkins L, Bitner-Glindzicz M: Connexin 26 as a cause of deafness in East London Bangladeshi population. **Royal Society of Medicine, London**, March 2005.

Bajaj Y, Sirimanna T, Albert D, Quader P, Jenkins L, Bitner-Glindzicz M. Causes of deafness in Bangladeshi Children. **Otolaryngologic Research Society, London**, September 2005.

Bajaj Y, Sirimanna T, Albert DM, Qadir P, Loughlin S, Jenkins L, Bitner-Glindzicz M: Is Connexin 26 a common cause of deafness in East London Bangladeshis? **University College London, London**, March 2005.

Bajaj Y, Sirimanna T, Albert DM, Qadir P, Loughlin S, Jenkins L, Bitner-Glindzicz M: Is Connexin 26 a common cause of deafness in East London Bangladeshis? **Institute of Child Health, London**, November 2004.

10.3 Information Leaflets about Research

10.3.1 Information sheet for adults

ADULT INFORMATION SHEET

GENETIC BASIS OF DEAFNESS IN EAST LONDON BANGLADESHI/ASIAN PEOPLE

AIMS

We want to find the causes of deafness in children and young people in East London and to see how many people have inherited deafness.

WHY IS THE STUDY BEING DONE?

In East London three times more children are born with deafness compared to other parts of the UK. In Tower Hamlets six times more Bangladeshi children have deafness than in Caucasian children. We don't know why this is. It may be because deafness is inherited in some families.

Some people have other relatives with deafness, so the cause of deafness in these people is very likely to be inherited. But most deaf people don't have other family members who are deaf. However, research has shown that where the deafness is more severe and starts from birth, deafness can still be inherited even when no one else in the family is deaf. This is because the deaf person has inherited something from both their mother's and their father's side of the family i.e. have a double dose of a factor that causes deafness.

We want to find these factors which cause inherited deafness in Bangladeshis.

HOW IS THE STUDY TO BE DONE?

To do this, we need to study as many deaf Bangladeshi/Asian individuals as possible in whom the cause of deafness is not known and so we are asking for your help. We would like to know how many people there are in your family and who is deaf and who is hearing. We would like you and you parents to give a blood sample (and sometimes brothers and sisters as well even though they may be hearing so that we can compare what has been inherited from parents). We will only use your blood for deafness research and not for anything else.

If several people in your family are deaf we will ask if we can contact these relatives to help with the research and give a blood sample. Your relatives might prefer to have their blood taken by their GP if they live far away.

We also need information about the severity of your hearing loss and we will need to look at your medical notes to check results of blood & urine tests and X rays. You won't need any extra tests other than normally done in an audiology or genetic clinic

ARE THERE ANY RISKS AND DISCOMFORTS?

Having a blood sample taken may be slightly painful so we will use a special cream to numb the skin when we take a blood sample from you.

We understand that some people might not want to know that their deafness is inherited. If you don't want to know, please tell us, and we will not give you any results from the study. Your results will be kept confidential. We hope that as many families as possible will want to help us find out more about why so many Bangladeshis are deaf even if they don't want results for themselves.

WHAT ARE THE POTENTIAL BENEFITS?

This study will not cure your deafness.

If we can easily find out the cause of inherited deafness in Bangladeshis, then in future when a Bangladeshi person/child is found to be deaf, he/she will not have to have as many tests to try and find the cause of deafness (blood tests, urine tests and special scans). Also we hope that we would be able to give better information to you about severity of your deafness as you grow older. This information might also help the audiology team looking after you to choose the best treatment for that type of deafness (eg hearing aid or cochlear implant or either).

WHO WILL HAVE ACCESS TO THE CASE RECORDS?

Only the research team working on the project and a person from the hospital whose job it is to make sure that the research is done properly (a member of the Research Ethics Committee).

WHAT ARE THE ARRANGEMENTS FOR COMPENSATION?

This project has been checked by the Health authority/Hospital and we do not think it is dangerous for you. However, if there is an unexpected problem we want you to know what you can do if any harm did come to you.

You could go to court to claim compensation. You would need to show that the problem happened because it was the hospital's fault.

DO I HAVE TO TAKE PART IN THE STUDY?

No, not if you don't want to. You will still have the same treatment in the clinic/hospital.

WHO DO I SPEAK TO IF PROBLEMS ARISE?

If you have a complaint about this research, first, please talk to the researcher. If this does not help, please contact the Chairman of the Research Ethics Committee, by post at the Research and Development Office, Institute of Child Health,
, or if urgent, by telephone on and the office will arrange for you to talk to him.

RESEARCHER WHO WILL HAVE CONTACT WITH THE FAMILY

Mr. Yogesh Bajaj, Dr. Maria Bitner-Glindzicz,

DETAILS OF HOW TO CONTACT THE RESEARCHER

Mr. Yogesh Bajaj:

or Dr. Maria Bitner-Glindzicz:

Or by post at Unit of Clinical and Molecular Genetics, Institute of Child Health,

10.3.2 Information sheet for parents

PARENTS INFORMATION SHEET

GENETIC CAUSES OF DEAFNESS IN EAST LONDON BANGLADESHI/ASIAN CHILDREN

AIMS

We want to find the causes of deafness in children and young people in East London and to see how many children have inherited deafness.

WHY IS THE STUDY BEING DONE?

In East London 3 times more children are born with deafness compared to other parts of the UK. In Tower Hamlets six times more Bangladeshi children have deafness than Caucasian children. We don't know why this is. It may be because deafness is inherited in some families.

Some children have other relatives with deafness, so the cause of deafness in these children is very likely to be inherited. But most deaf children don't have other family members who are deaf. However, research has shown that where the deafness is more severe and starts from birth, deafness can still be inherited even when no one else in the family is deaf. This is because the deaf child has inherited something from both their mother's and their father's side of the family i.e. have a double dose of a factor that causes deafness.

We want to find these factors which cause inherited deafness in Bangladeshi children.

HOW IS THE STUDY TO BE DONE?

To do this, we need to study as many deaf Bangladeshi/Asian children as possible in whom the cause of deafness is not known and so we are asking for your help. We would like to know how many people there are in your family and who is deaf and who is hearing. We would like the deaf child and his/her parents to give a blood sample (and sometimes brothers and sisters as well even though they may be hearing so that we can compare what has been inherited from parents). We will only use the blood for deafness research and not anything else.

If several people in your family are deaf we will also ask if we can contact these relatives to help with the research and give a blood sample. Your relatives might prefer to have their blood taken by their GP if they live far away.

We also need information about the severity of your child's deafness and we will need to look at their medical notes to check results of blood & urine tests and X-rays. Your child won't need any extra tests other than those normally done in an audiology or genetic clinic

ARE THERE ANY RISKS AND DISCOMFORTS?

Having a blood sample taken may be slightly painful so we will use a special cream to numb the skin when we take a blood sample from your child.

Some people might not want to know that their child's deafness is inherited. If you don't want to know, please tell us, and we will not give you any results from the study. Your child's results will be kept confidential. We hope that as many families as possible will want to help us find out more about why so many Bangladeshi children are deaf even if they don't want results for themselves.

WHAT ARE THE POTENTIAL BENEFITS?

This study will not cure your child's deafness.

If we can easily identify which Bangladeshi children have inherited deafness, then in future when a Bangladeshi child is found to be deaf, he/she won't have to have as many tests to try and find the cause of deafness (blood tests, urine tests and special scans). Also we hope that we would be able to give better information to parents about severity of their child's deafness as the child gets older. This information might also help the audiology team looking after the child to choose the best treatment for that type of deafness (eg hearing aid or cochlear implant or either)

WHO WILL HAVE ACCESS TO THE CASE RECORDS?

Only the research team working on the project and a person from the hospital whose job it is to make sure that the research is done properly (member of the Research Ethics Committee).

WHAT ARE THE ARRANGEMENTS FOR COMPENSATION?

The Health Authority/ Hospital has checked this project and we do not think it is dangerous for you or your child. However, if there is an unexpected problem we want you to know what you can do if any harm did come to your child.

You could go to court to claim compensation. You would need to show that the problem happened because it was the Hospital's fault.

DO I HAVE TO TAKE PART IN THE STUDY?

No, not if you don't want to. Your child will still have the same treatment in the clinic/hospital.

WHO DO I SPEAK TO IF PROBLEMS ARISE?

If you have a complaint about this research, first, please talk to the researcher. If this does not help, please contact the Chairman of the Research Ethics Committee, by post at the Research and Development Office, Institute of Child Health,
, or if urgent, by telephone on and the office will arrange for you to talk to him/her.

RESEARCHER WHO WILL HAVE CONTACT WITH THE FAMILY

Mr. Yogesh Bajaj , Dr. Maria Bitner-Glindzicz,

DETAILS OF HOW TO CONTACT THE RESEARCHER

Mr. Yogesh Bajaj: , or Dr. Maria Bitner-Glindzicz:

Or by post at Unit of Clinical and Molecular Genetics, Institute of Child Health,

10.3.3 Information sheet for children

Information for Children

We are asking you to help in a study to look for the causes of poor hearing in some children.

1. Aim of the study:

We would like to try and find out why some children have poor hearing.

2. Why is this study being done?

We do not know why some Bengali/Asian children have poor hearing. We want to try and work out why this happens.

3. How is this study being done?

When you come to the clinic you and your parents will be asked whether you would like to help with the study. If you want to help we will ask your parents about all the other people in your family and will ask you, your parents and your brothers and sisters to give a blood sample. Sometimes we might ask other family members to help us (an aunt, uncle, cousin or grandparents).

4. What are the dangers?

Having a blood sample taken is not at all dangerous but may be uncomfortable. If you wish we will use a cream which numbs the skin so that you will hardly feel anything.

5. Who will know about your help with this study?

Only the doctor involved in the study and your family doctor(GP). We do not put any extra information in the hospital notes.

6. What do we hope to find out?

We are trying to understand what causes some children to be born with hearing loss but it may take us a long time. However, we hope that in the future we will understand more about the problem and this will be helpful to other children and their families.

7. Do you have to take part in the study?

No, you do not have to help with the study if you don't want to, and nobody will mind.

8. If you are unhappy with the study, let your parents know as they have information on how to get in touch with us.

10.3.4 Information sheet for General Practitioners

DEAFNESS IN EAST LONDON BENGALI PEOPLE

Genetic causes are responsible for a large proportion of deafness in childhood even where there is no one else in the family with deafness.

In East London three times as many children are born with hearing problems, as compared to other parts of the UK. In Bengali children in Tower Hamlets six times as many children have hearing problems than in Caucasians. This may be because genes causing deafness might be common in East London Asians, especially people whose families have come from Bangladesh. We think that the main cause of deafness in many children is due to some unknown gene, which is being passed down the generations. It probably causes deafness only when two such genes are present in one individual one inherited from each parent. We are hoping to find that gene.

Although some people are known to have a family history of deafness, many others know of no other relatives in their family who suffer from this problem. Nevertheless, research has shown that even where there is no family history, many cases have a genetic cause, particularly where the hearing loss is more severe and is of early onset in life and no other cause for the deafness has been found.

We hope that if we can find out the gene causing deafness in a child then the child will not have to go through all the other tests that are done to find the cause of deafness (blood tests, urine tests and special scans). Also if we know which genes can cause deafness in Bengalis/Asians we may be able to offer genetic testing of the commoner genes for families who request it (parents and relatives of deaf individuals).

In order to do this, we need to study as many deaf Bengali/Asian individuals as possible in whom the cause of deafness is not known.

We need a blood sample to search for genes causing deafness.

For some families the gene responsible for their deafness may be identified within the next few years and testing could be offered to other family members to see if they carry the gene.

DETAILS FOR FURTHER INFORMATION

Mr. Yogesh Bajaj:

Dr. Maria Bitner-Glindzicz:

Or by post at

Departments of Clinical and Molecular Genetics,
Institute of Child Health,

10.3.5 Information leaflet for parents in Bengali

বাবা-মায়ের জন্য তথ্য শীট

পূর্ব লন্ডনের বাংলাদেশী/এশিয়ান ছেলেমেয়ের বধির হওয়া বা কানে না শোনার জিনগত কারণ

10.3.6 Information leaflet for adults in Bengali

প্রাপ্তবয়স্কদের জন্য তথ্যশীট

পূর্ব লন্ডনের বাংলাদেশী/এশিয়ান লোকজনের বধির হওয়া বা কানে না শোনার জিনগত ভিত্তি

10.4 Letters to patients and physicians

10.4.1 Invitation for Research

Subject: Research on causes of hearing loss in Bangladeshis/ Asians in East London.

Dear Sir/ Madam,

We are writing to you about deafness in Bangladeshis/Asians in East London. The rate of deafness in Bangladeshis is six times more than the national average. We are starting a research project to find out the cause for this.

I am writing this letter to invite you to join this research, to find the causes of deafness in Bangladeshis/ Asians in East London. Your help will mean giving us medical details about your child's/your hearing loss and giving a blood sample.

If you want to find out more about this research, kindly give a ring to Dr Yogesh Bajaj on mobile _____ or at _____. If you want to help with the research kindly fill out the slip enclosed and send it back in the prepaid envelope.

I hope you will help us to find the reason for increased rate of deafness in the community.

Thanks.

Yours faithfully,

Mr Yogesh Bajaj,

Dr M Bitner Glindzicz

Reply Slip

Your Name:

Child's Name:

Child's Date of birth:

Address:

Donald Winnicot Number:

(if known)

Other hospital number:

Telephone:

Would you prefer to be seen in the clinic at Donald Winnicot Centre or at your home:

10.4.2 Invitation for Research in Bengali

বিষয়: পূর্ব লভনে বাংলাদেশী/এশিয়ানদের মধ্যে কানে কম শোনা বা বধিরতার কারণ সম্পর্কে গবেষণা

প্রিয় জনাব/জনাব,

আমরা পূর্ব লভনে বাংলাদেশী/এশিয়ানদের বধিরতা বা কানে কম শোনার সম্পর্কে আপনার কাছে লিখছি। বাংলাদেশীরা মধ্যে বধিরতার হার জাতীয় গড় বধিরতার হারের চেয়ে ছয়গুন বেশি। এর কারণ বুঝে বের করার জন্য আমরা একটি গবেষণা প্রকল্প শুরু করছি।

আমি এই চিঠি লিখছি এই গবেষণায় আপনাদের যোগ দিতে আমন্ত্রণ জানানোর জন্য, যাতে পূর্ব লভনে বাংলাদেশী/এশিয়ানদের বধিরতার কারণ বুঝে বের করা যায়। আপনার এতে সাহায্য করার মানে হবে এই যে, আপনি আপনার বাচ্চা/আপনার কানে শোনার সমস্যা বা বধিরতার বিবরণ দেবেন এবং একটা রক্তের নমুনাও দেবেন।

আপনি যদি এই গবেষণা সম্পর্কে আরো জানতে চান, তাহলে ডঃ ইয়োগেশ বাজাজ-কে এই মোবাইল নম্বরে ফোন করুন -। এই

। বীপ করতে বলুন যদি আপনি এই গবেষণায় সাহায্য করতে চান তাহলে দয়া করে সাথে দেওয়া এই কণজটা ওঠে করে প্রিন্টেইট এনভেলোপে ফেরত পাঠান।

আমি আশা করছি যে এই কর্মউনিটিতে বধিরতার হার বেড়ে যাওয়ার কারণ বুঝে বের করতে আপনারা আমাদের সাহায্য করবেন।

ধন্যবাদ

আপনাদের বিশ্বস্ত

ডঃ ইয়োগেশ বাজাজ, ডঃ টিনি সিরিয়াননা, ডঃ এম বিটনার পরিচালিত

জবাবের পত্র

আপনার নাম

বাচ্চার নাম

ঠিকানা:

বাচ্চার জন্মতারিখ:

চোনাস উইনিংকট নম্বর:

(যদি জানা থাকে)

অন্য হাসপাতাল নম্বর:

টেলিফোন:

আপনি কি চোনাস উইনিংকট সেন্টারের ড্রিমকে না আপনার বাড়িতে দেখাতে চাইবেন?

1521267-04 Bengali-Recruitment letter

10.4.3 Appointment letter

Dear Sir/ Madam,

You have been given an appointment to see Dr Yogesh Bajaj, Registrar ENT, at on
at the Donald Winnicot Centre, Coate Street, London. You will be seen in the audiology
department at second floor. The appointment will last approximately 30-60 minutes.
Kindly let the audiology department or know when you have arrived.

If you want to cancel your appointment, please call Dr Bajaj

Thanks

Dr Yogesh Bajaj

10.4.4 Letter to General Practitioner

Dear Doctor,

We are conducting a research to look for gene/s causing deafness in East London Bangladeshi population. In East London three times more children are born with deafness compared to other parts of the UK. In Tower Hamlets six times more Bangladeshi children have deafness than in Caucasian children. We don't know why this is? It may be because deafness is inherited in some families.

Your patient has kindly agreed to take part in our research into the genetic basis of deafness.

We would like to take a detailed history and ask the patient and their parents to give a blood sample (and sometimes brothers and sisters as well even though they may be hearing).

If we can easily find out the cause of inherited deafness in Bangladeshis, then in future when a Bangladeshi person/child is found to be deaf, he/she will not have to have as many tests to try and find the cause of deafness.

The research is being conducted under the guidance of Dr M Bitner Glindzicz, Consultant Genetics, Institute of Child Health. I enclose a copy of the patient information sheet for your records.

Yours faithfully,

Mr Y. Bajaj, MRCS, DLORCS
Research Fellow ENT

10.4.5 Letter of thanks to patients

Re: Bangladeshi children hearing loss project

Dear Mr & Mrs

I am writing this letter to thank you for coming to the audiology clinic at Donald Winnicot Centre and agreeing to participate in the Bangladeshi children hearing loss project. I really appreciate your help which will be beneficial for the Bengali community and your child in the future. I will be writing to you in due course of time to inform you of our progress in this study.

Thanks once again.

Yours sincerely,

Dr Yogesh Bajaj
Registrar ENT

10.4.6 Letter to patients about results

Dear Mr & Mrs

I am writing to you about our meeting at the Donald Winnicot Centre on 17/2/2004. As you would remember I had taken some blood from your child at that appointment. I have got results from that blood sample.

After genetic testing at Great Ormond Street hospital we have found that your child is positive for Connexin 26 gene. The most likely cause of deafness in your child is due to a gene called "Connexin 26".

You will be receiving a letter in the post from Dr M Bitner Glindzicz, Consultant Geneticist at Great Ormond Street Hospital inviting you to see her for genetic counselling and further advice.

If I can be of any further help, do not hesitate to contact me or

Thanks

Yours sincerely,

Dr Yogesh Bajaj
Research Fellow, ENT
Great Ormond Street Hospital

Cc:

10.5 Data Collection Sheet

Hearing loss Research

Case Number:

Title:

Surname:

First Name:

DOB:

Age:

Hospital Number:

Father's Name:

Mother's Name:

Address:

Contact number:

G.P.:

Consultant:

School:

History

Present History

Duration of decreased hearing:

When was hearing loss first suspected?

How?

Did the child undergo neonatal hearing screening?

When confirmed?

Was there a delay in the diagnosis?

If yes, factors leading to the delay in diagnosis:

Has it changed/progressed?

Hearing Aids given:

Re, audio tape:

How does child/person communicate?

Is sign language used at home?

Is the sign language acceptable to parents?

Languages spoken at home:

Child's first language:

Originally from:

Severity of deafness(Audiogram):

Date								
	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
Hearing Level								
Frequency Range								
Audiogram Shape								
Tympanogram								
Date								
	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
Hearing Level								
Frequency Range								
Audiogram Shape								
Tympanogram								

Progression of deafness over time:

Does it fluctuate?

Ear discharge:

Tinnitus:

Vertigo:

Speech:

Any other ENT problems:

Any other current problems:

Diagnosis:

Cause of SN hearing loss in this patient:

How has this been ascertained: Clinical/molecular/lab test:

Treatment planned:

Past History

Antenatal History:

Viral illnesses in pregnancy:

Perinatal History:

Birth Weight:

Gestation of pregnancy:

Delivery:

Postnatal history:

Jaundice:

Meningitis:

Developmental History:

Age of head control:

Age of sitting:

Age of walking:

Any past medical problems:

Any past surgeries:

Family History

Consanguinity:

Family history of deafness: Siblings

Parents

Grand parents

Cousins

Any other illnesses in the family:

Family Tree:

Attitude:

Are the child/ parents interested in knowing the cause of deafness?

Why?

Did a deaf child in the family affect their completing the family?

If we found a gene causing deafness in them/their child do they think other relatives will be interested in genetic testing?

Would they want genetic testing for family planning in future?

Views on prenatal testing considering the risk of miscarriage:

Would they continue with the pregnancy if child found deaf on prenatal testing:

Examination

General Physical Examination:

Facial Features:

Ears:

External ear shape:

Pits/Tags:

External canal:

Tympanic membrane:

Nose:

Throat:

Neck:

Fistulae:

Goitre:

Any other abnormalities:

Investigations done:

Patient/Child:

Blood Tests:

Radiology:

Family hearing assessments:

Siblings:

Mother:

Father:

Grandmother:

Grandfather:

Other relatives:

Genetic mapping:

(Blood collected)

Patient/ Child:

Siblings:

Mother:

Father:

Grandmother:

Grandfather:

Other relatives:

10.6 Census Data

2001 CENSUS TABLE S101 SEX AND AGE BY ETHNIC GROUP - HACKNEY

Table population: All people

Geographical level: England and Wales to Ward

		ALL PEOPLE	Asian or Asian British: Bangladeshi
00AM Hackney	ALL PEOPLE	202,820	5,972
00AM Hackney	0 to 4	16,752	792
00AM Hackney	5 to 7	8,466	441
00AM Hackney	8 to 9	5,700	252
00AM Hackney	10 to 14	14,082	685
00AM Hackney	15	2,430	159
00AM Hackney	16 to 17	5,282	294

Cells in this table have been randomly adjusted to avoid the release of confidential data.

2001 CENSUS TABLE S101 SEX AND AGE BY ETHNIC GROUP - TOWER HAMLETS

Table population: All people

Geographical level: England and Wales to Ward

		ALL PEOPLE	Asian or Asian British: Bangladeshi
00BG Tower Hamlets	ALL PEOPLE	196,100	65,553
00BG Tower Hamlets	0 to 4	15,177	8,739
00BG Tower Hamlets	5 to 7	8,137	4,915
00BG Tower Hamlets	8 to 9	5,228	2,957
00BG Tower Hamlets	10 to 14	13,708	8,032
00BG Tower Hamlets	15	2,640	1,525
00BG Tower Hamlets	16 to 17	5,413	3,154

Cells in this table have been randomly adjusted to avoid the release of confidential data.

11 References

References

- Abe, S., P. M. Kelley, W. J. Kimberling, and S. Usami. 2001. Connexin 26 gene (GJB2) mutation modulates the severity of hearing loss associated with the 1555A->G mitochondrial mutation. *Am.J.Med.Genet.* **103**:334-338.
- Abe, S., S. Usami, H. Shinkawa, P. M. Kelley, and W. J. Kimberling. 2000. Prevalent connexin 26 gene (GJB2) mutations in Japanese. *Journal of Medical Genetics* **103**:334-338.
- Ahmad, S., S. Chen, J. Sun, and X. Lin. 2003. Connexin 26 and 30 are co-assembled to form gap junctions in the cochlea of mice. *Biochem.Biophys.Res.Commun.* **307**:362-368.
- Alvarez, A., I. del Castillo, M. Villamar, L. A. Aguirre, A. Gonzalez-Neira, A. Lopez-Nevot, M. A. Moreno-Pelayo, and F. Moreno. 2005. High prevalence of the W24X mutation in the gene encoding connexin-26 (GJB2) in Spanish Romani (gypsies) with autosomal recessive non-syndromic hearing loss. *American Journal of Medical Genetics* **137**:255-258.
- Armitage, I. M., J. P. Burke, and J. T. Buffin. 1995. Visual impairment in severe and profound sensorineural deafness. *Arch.Dis.Child.* **73**:53-56.
- Baroch, K. A. 2003. Universal newborn hearing screening: fine-tuning the process. *Curr.Opin.Otolaryngol Head Neck Surg* **11**:424-427.
- Bathey, J. F., Jr. 2000. A genetic approach to understanding inner ear function. *The Journal of Clinical Investigation* **106**:1431-1432.
- Bauer, P. W., A. E. Geers, C. Brenner, J. S. Moog, and R. J. H. Smith. 2003. The effect of GJB2 allele variants on performance after cochlear implantation. *Laryngoscope* **113**:2135-2140.
- Bitner-Glindzicz, M. 2002. Hereditary deafness and phenotyping in humans. *Br.Med.Bull.* **63**:73-94.
- Brobby, G. W., B. Muller-Myhsok, and R. D. Horstmann. 1998. Connexin 26 R143W mutation associated with recessive nonsyndromic sensorineural deafness in Africa. *New England Journal of Medicine* **338**:548-550.

- Brunger, J. W., G. S. Murray, M. O'Riordan, A. L. Matthews, R. J. Smith, and N. H. Robin. 2000a. Parental attitudes toward genetic testing for pediatric deafness. *Am.J.Hum.Genet.* **67**:1621-1625.
- Brunger, J. W., G. S. Murray, M. O'Riordan, A. L. Matthews, R. J. H. Smith, and N. H. Robin. 2000b. Parental attitudes toward genetic testing for paediatric deafness. *American Journal of Human Genetics* **67**:1621-1625.
- Castillo, I. d., M. Villamar, M. A. Moreno-Pelayo, F. J. d. Castillo, A. Alvarez, D. Telleria, I. Menendez, and F. Moreno. 2002. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *New England Journal of Medicine* **346**:243-249.
- Chang, E. H., G. VanCamp, and R. J. Smith. 2003. The role of connexins in human disease. *Ear and Hearing* **24**:314-317.
- Cohn, E. S., and P. M. Kelley. 1999. Clinical phenotype and mutations in Connexin 26(DFNB1/GJB2), the most common cause of childhood hearing loss. *American Journal of Medical Genetics* **89**:130.
- Cohn, E. S., P. M. Kelley, T. W. Fowler, M. P. Gorga, D. M. Lefkowitz, H. J. Kuhen, G. B. Schaefer, L. S. Gobar, F. J. Hahn, D. J. Harris, and W. J. Kimberling. 1999. Clinical studies of families with hearing loss attributable to mutations in the Connexin 26 gene (GJB2/DFNB1). *Pediatrics* **103**:546.
- Cohne, O. P., J. E. Fischgrund, and R. Redding. 1990. Deaf children from ethnic linguistic and racial minority backgrounds: an overview. *American Annals of the Deaf* **135**:67-73.
- Cryns, K., E. Orzan, A. Murgia, P. L. M. Huygen, F. Moreno, I. d. Castillo, G. P. Chamberlin, H. Azaiez, S. Prasad, R. A. Cucci, E. Leonardi, R. L. Snoeckx, P. J. Govaerts, P. H. Van de Heyning, C. M. Van den Heyning, R. J. H. Smith, and G. Van Camp. 2004. A genotype-phenotype correlation for GJB2 (Connexin 26) deafness. *Journal of Medical Genetics* **41**:147-154.
- Cullen, R. D., C. A. Buchman, C. J. Brown, B. J. Copeland, C. Zdanski, Pillsbury H.C.3rd., and C. G. Shores. 2004. Cochlear implantation for children with GJB2-related deafness. *Laryngoscope* **114**:1415-1419.
- Dalzell, L., M. Orlando, M. MacDonald, A. Berg, M. Bradley, and A. Cacace. 2000. The New York State universal newborn hearing screening demonstration project: ages of hearing loss identification, hearing aid fitting, and enrolment in early intervention. *Ear and Hearing* **21**:118-130.
- Davis, A., J. Bamford, and J. Stevens. 2001a. Performance of neonatal and infant hearing screens: sensitivity and specificity. *British Journal of Audiology* **35**:3-15.

- Davis, A., J. Bamford, I. Wilson, T. Ramkalawan, M. Forshaw, and S. Wright. 1997. A critical review of the role of the role of neonatal hearing screening in the detection of congenital hearing impairment. *Health Technology Assessment* 1:1-176.
- Davis, A., and G. Mencher. 2002. Epidemiology of permanent childhood hearing impairment. Pages 65-90 *in* V. E. Newton editor. *Paediatric Audiological Medicine*. Whurr Publishers, London.
- Davis, A., S. Wood, and R. Healy. 1995. Risk factors for hearing disorders: Epidemiological evidence of change over time in the UK. *J.Am.Acad.Audiol.* 6:365-370.
- Davis, A., C. Yoshinaga-Itano, and S. Hind. 2001b. Commentary: Universal newborn hearing screening: implications for coordinating and developing services for deaf and hearing impaired children. *BMJ* 323:536-540.
- del Castillo, I., M. A. Moreno-Pelayo, F. J. del Castillo, Z. Brownstein, S. Marlin, Q. Adina, D. J. Cockburn, A. Pandya, K. R. Siemering, G. P. Chamberlin, E. Ballana, W. Wuyts, A. T. Maciel-Guerra, A. Alvarez, M. Villamar, M. Shohat, D. Abeliovich, H. H. Dahl, X. Estivill, P. Gasparini, T. Hutchin, W. E. Nance, E. L. Sartorato, R. J. Smith, G. Van Camp, K. B. Avraham, C. Petit, and F. Moreno. 2003. Prevalence and evolutionary origins of the del (GJB6-D13S1830) mutation in the DFNB1 locus in hearing impaired subjects: a multicenter study. *American Journal of Human Genetics* 73:1452-1457.
- del Castillo, I., M. Villamar, M. A. Moreno-Pelayo, F. J. del Castillo, A. Alvarez, D. Telleria, I. Menendez, and F. Moreno. 2002. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *New England Journal of Medicine* 346:243-246.
- Denoyelle, F., G. Lina-Granade, H. Plauchu, R. Bruzzone, H. Chaib, F. Levi-Acobas, D. Weil, and C. Petit. 1998. Connexin 26 gene linked to a dominant deafness. *Nature* 393:319-320.
- Denoyelle, F., S. Marlin, D. Weil, L. Moatti, P. Chauvin, E. N. Garabedian, and C. Petit. 1999. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling. *Lancet* 353:1298-1303.
- Denoyelle, F., D. Weil, and M. A. Maw. 1997. Prelingual deafness: High prevalence of a 30delG mutation in the Connexin 26 gene. *Human Molecular Genetics* 6:2173-2177.
- Dixon, M. J., J. Gazzard, S. S. Chaudhary, N. Sampson, B. A. Schulte, and K. P. Steel. 1999. Mutation of the Na-K-Cl co-transporter gene *Slc12a2* results in deafness in mice. *Hum.Mol.Genet.* 8:1579-1584.

- Durkin, M. S., N. Z. Khan, L. L. Davidson, S. Huq, S. Munir, E. Rasul, and S. S. Zaman. 2000. Prenatal and postnatal risk factors for mental retardation among children in Bangladesh. *American Journal of Epidemiology* **152**:1024-1033.
- Egeli, E., G. Cicekci, F. Silan, O. Ozturk, U. Harputluoglu, A. Onur, A. Egeli, and A. Yildiz. 2003. Etiology of deafness at the Yeditepe School for the deaf in Istanbul. *International Journal of Pediatric Otorhinolaryngology* **67**:467-471.
- Estivill, X., P. Fortina, S. Surrey, R. Rabionet, S. Melchionda, L. D'Agruma, E. Mansfield, E. Rappaport, N. Govea, M. Mila, L. Zelante, and P. Gasparini. 1998. Connexin 26 mutations in sporadic and inherited sensorineural deafness. *Lancet* **351**:394-398.
- Flock, A., A. Bretscher, and K. Weber. 1982. Immunohistochemical localisation of several cytoskeletal proteins in inner ear sensory and supporting cells. *Hear.Res.* **7**:75-89.
- Forge, A., and T. Wright. 2002. The molecular architecture of the inner ear. *Br.Med.Bull.* **63**:5-24.
- Fortnum, H. M. 2003. Epidemiology of Permanent Childhood Hearing impairment: Implications for neonatal hearing screening. *Audiological Medicine* **1**:155-164.
- Fortnum, H. M., A. Q. Summerfield, D. H. Marshall, A. C. Davis, and J. M. Bamford. 2001. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire based ascertainment study. *BMJ* **323**:536-540.
- Fraser, G. R. 1976. The causes of profound deafness in childhood. *in* Baltimore. John Hopkins University Press.
- Fukushima, K., K. Sugata, N. Kasai, S. Fukuda, R. Nagayasu, N. Toida, N. Kimura, T. Takishita, M. Gunduz, and K. Nishizaki. 2002. Better speech performance in cochlear implant patients with GJB2 related deafness. *International Journal of Pediatric Otorhinolaryngology* **62**:151-157.
- Gasparini, P., R. Rabionet, G. Barbujani, S. Melchionda, M. Petersen, K. Brondum-Nielsen, A. Metspalu, E. Oitmaa, M. Pisano, P. Fortina, L. Zelante, and X. Estivill. 2000. High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG. *Eur.J.Hum.Genet.* **8**:19-23.
- Ghosh, M., R. Vijaya, and M. Kabra. 2004. Genetics of deafness in India. *Indian Journal of Pediatrics* **71**:531-533.
- GLA Data mangement and analysis group. 2001 Census profiles: Bangladeshis in London. DMAG Breifing 2004. 2004.

Ref Type: Report

- Gorlin R. J., H. V. Toriello, and M. M. Cohen. 1995. Hereditary hearing loss and its syndromes. Oxford University Press, Oxford.
- Green, G. E., R. F. Mueller, E. S. Cohn, K. B. Avraham, M. Kanann, and R. J. H. Smith. 2003. Audiological manifestations and features of Connexin 26 deafness. *Audiological Medicine* 1:5.
- Green, G. E., D. A. Scott, J. M. McDonald, H. F. B. Teagle, B. J. Tomblin, L. J. Spencer, G. G. Woodworth, J. F. Knutson, B. J. Gantz, V. C. Sheffield, and R. J. H. Smith. 2002. Performance of cochlear implant recipients with GJB2 related deafness. *American Journal of Medical Genetics* 109:167-170.
- Green, G. E., D. A. Scott, J. M. McDonald, G. G. Woodworth, V. C. Sheffield, and R. J. H. Smith. 1999. Carrier rates in the midwestern United States for GJB2 mutations causing inherited deafness. *JAMA* 281:2211-2216.
- Green, G. E., R. J. Smith, J. P. Bent, and E. S. Cohn. 2000. Genetic testing to identify deaf newborns. *JAMA* 284:1245.
- Guilford, P., S. B. Arab, J. Blanchard, J. Levilliers, J. Weissenbach, and A. Belkahia. 1994. A non-syndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. *Nature Genetics* 6:24-28.
- Guilford, P., S. BenArab, S. Blanchard, J. Levilliers, J. Weissenbach, and A. Belkahia. 2000. Joint Committee on Infant Hearing; American Academy of Audiology; American Academy of Pediatrics; American Speech-Language-Hearing Association; Directors of Speech and Hearing Programs in State Health and Welfare Agencies. Year 2000 position statement: principles and guidelines for early hearing detection and intervention programs. *Pediatrics* 106:798-817.
- Haggard, M. P., B. McCormick, M. Gannon, and H. Spencer. 1992. The paediatric otological caseload resulting from improved screening in the first year of life. *Clinical Otolaryngology* 17:34-43.
- Hamelmann, C., G. K. Amedofu, K. Albrecht, B. Muntau, A. Gelhaus, G. W. Brobby, and R. D. Horstmann. 2001. Pattern of Connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. *Human Mutation* 18:84-85.
- Harris, A. L. 2001. Emerging issues of connexin channels: biophysics fills the gap. *Q.Rev.Biophys.* 34:325-472.
- Hone, S. W., and R. J. Smith. 2002. Medical evaluation of pediatric hearing loss: laboratory, radiographic and genetic testing. *Otolaryngol.Clin.North Am.* 35:751-764.
- <http://webhost.ua.ac.be/hhh>. Hereditary hearing loss homepage. 2005.

Ref Type: Generic

Janzen, V. D., and D. Schaefer. 1984. Etiology of deafness in Robarts school for the deaf. *Journal of Otolaryngology* **13**:47-48.

Kelsell, D. P., W. L. Di, and M. J. Houseman. 2001. Connexin mutations in skin disease and hearing loss. *American Journal of Human Genetics* **68**:559-568.

Kelsell, D. P., J. Dunlop, H. P. Stevens, N. J. Lench, J. N. Liang, G. Parry, R. F. Mueller, and I. M. Leigh. 1997. Connexin 26 mutations in hereditary nonsyndromic sensorineural deafness. *Nature* **387**:80-83.

Kempson E. Overcrowding in Bangladeshi households-a case study of Tower Hamlets. 863, 1-31. 1999. Policy Study Institute.

Ref Type: Report

Kennedy, C., L. Kimm, R. Thornton, and A. Davis. 2000. False positives in universal neonatal screening for permanent childhood hearing impairment. *Lancet* **356**:1903-1904.

Kikuchi, T., R. S. Kimura, D. L. Paul, and J. C. Adams. 1995. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. *Anat.Embryol.* **191**:101-118.

Kubisch, C., B. C. Schroeder, T. Friedrich, B. Lütjohann, A. El-Amraoui, S. Marlin, C. Petit, and T. J. Jentsch. 1999. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell* **96**:437-446.

Kudo, T., K. Ikeda, T. Oshima, S. Kure, M. Tammaesang, S. Prasansuk, and Y. Matsubara. 2001. GJB2 (connexin 26) mutations and childhood deafness in Thailand. *Otology & Neurotology* **22**:858-861.

Kuijpers, W., E. L. Tonnaer, T. A. Peters, and F. C. Ramaekers. 1991. Expression of intermediate filament proteins in the mature inner ear of the rat and guinea pig. *Hear.Res.* **52**:133-146.

Kumar, N. M., and N. B. Gilula. 1996. The gap junction communication channel. *Cell* **84**:381-388.

Lee, M. P., J. D. Ravenel, H. Ren-Ju, R. L. T. Lawrence, D. B. Ronald, A. B. Sheri, A. B. Sheri, J. L. B. Tracy, L. Charles, F. Howard, G. Melissa, G. W. Hua, A. Pedram, r. g. James, and J. C. F. Robert. 2000. Targeted disruption of the Kvlqt1 gene causes deafness and gastric hyperplasia in mice. *J.Clin.Invest.* **106**:1447-1455.

Lench, N., M. Houseman, V. Newton, G. Van Camp, and R. Mueller. 1998. Connexin-26 mutations in sporadic non-syndromal sensorineural deafness. *Lancet* **351**:415.

- Lerer, I., M. Sagi, E. Malamud, H. Levi, A. Raas-Rothschild, and D. Abeliovich. 2000. Contribution of Connexin 26 mutations to nonsyndromic deafness in Ashkenazi patients and the variable phenotypic effect of the mutation 167delT. *Am.J.Med.Genet.* **95**:53-56.
- Lerer, I., M. Sagi, Z. Ben-Neriah, T. Wang, H. Levi, and D. Abeliovich. 2001. A deletion mutation in GJB6 cooperating with GJB2 mutation in trans in non-syndromic deafness: a novel founder mutation in Ashkenazi Jews. *Human Mutation* **18**:460.
- Liu, X., and L. Xu. 1994. Nonsyndromic hearing loss: an analysis of audiograms. *Ann.Otol.Rhinol.Laryngol.* **103**:428-433.
- Löffler, J., D. Nekham, A. Hirst-Stadlmann, B. Gunther, H. J. Menzel, G. Utermann, and A. R. Janecke. 2001. Sensorineural hearing loss and the incidence of Cx 26 mutations in Austria. *Eur.J.Hum.Genet.* **9**:226-230.
- Lucas, D. 2002. Progressive hearing loss. Pages 269-293 in V. Newton editor. *Paediatric Audiological Medicine*. Whurr publishers, London.
- Maheshwari, M., R. Vijaya, M. Ghosh, S. Shastri, M. Kabra, and P. S. N. Menon. 2003. Screening of families with autosomal recessive non-syndromic hearing impairment (ARNSHI) for mutations in GJB2 gene: Indian scenario. *American Journal of Medical Genetics* **120A**:180-184.
- Mäki-Torkko, E. M., P. K. Lindholm, M. R. H. Väyrynen, J. T. Leisti, and M. J. Sorri. 1998. Epidemiology of moderate to profound childhood hearing impairments in Northern Finland. Any changes in ten years? *Scandinavian Audiology* **27**:95-103.
- Marazita, M. L., L. M. Ploughman, B. Rawlings, E. Remington, K. S. Arnos, and W. E. Nance. 1993. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. *Am.J.Med.Genet.* **46**:486-491.
- Martin, J., O. Bentzen, and J. Colley. 1981. Childhood deafness in the European Community. *Scand.Audiol.* **10**:165-174.
- Martinez, A., J. Linden, L. A. Schimmenti, and C. G. Palmer. 2003. Attitudes of the broader hearing, deaf and hard of hearing community toward genetic testing for deafness. *Genet Med* **5**:106-112.
- Martini, A., A. Prosser, M. Mazzoli, and M. Rosignoli. 1996. Contribution of age related factors to the progression of non-syndromic hereditary hearing impairment. *J Audiol Med* **5**:141-156.
- Matsushiro, N., K. Doi, Y. Fuse, K. Nagai, K. Yamamoto, T. Iwaki, T. Kawashima, A. Sawada, H. Hibino, and T. Kubo. 2002. Successful cochlear implantation in prelingual profound deafness resulting from the common 233delC mutation of the GJB2 gene in the Japanese. *Laryngoscope* **112**:255-261.

- McCormick, B. 1993. Behavioural hearing tests 6 months to 3;6 years. Pages 102-123 in *Paediatric Audiology, 0-5 years*. Whurr Publishers.
- McFarland, W. H., and F. B. Simmons. 1980. The importance of early intervention with severe childhood deafness. *Pediatric Annals* **9**:13-19.
- Middleton, A., J. Hewison, and R. F. Mueller. 1998. Attitudes of deaf adults toward genetic testing for hereditary deafness. *Am.J.Hum.Genet.* **63**:1175-1180.
- Middleton, A., J. Hewison, and R. F. Mueller. 2001. Prenatal diagnosis for inherited deafness: what is the potential demand? *Journal of Genetic Counselling* **10**:121.
- Moeller, M. P. 2000. Early intervention and language development in children who are deaf and hard of hearing. *Pediatrics* **106**:e43.
- Moore, W. G., J. A. Josephson, and G. W. Mauk. 1991. Identification of children with hearing impairments: a baseline survey in Volta. *Review* **93** 187-195.
- Morell, R. J., H. J. Kim, H. J. Hood, L. Goforth, K. Friderici, R. Fisher, G. Van Camp, C. I. Berlin, C. Oddoux, H. Ostrer, B. Keats, and T. B. Friedman. 1998. Mutations in the connexin 26 gene (GJB2) among Ashkenazi Jews with nonsyndromic recessive deafness. *New England Journal of Medicine* **339**:1500-1505.
- Morle, L., M. Bozon, N. Alloisio, P. Latour, A. Vandenberghe, H. Plauchu, I. Collet, P. Edery, J. Godet, and G. Lina-Granade. 2000. A novel C202F mutation in the connexin 26 gene (GJB2) associated with autosomal dominant isolated hearing loss. *Journal of Medical Genetics* **37**:368-370.
- Morton, N. E. 1991. Genetic epidemiology of hearing impairment. *Annals of New York-Academy of Science* **630**:16-31.
- Morton, R., V. Sharma, J. Nicholson, M. Broderick, and J. Poyser. 2002. Disability in children from different ethnic populations. *Child.Care.Health Dev.* **28**:87-93.
- Mueller, R. F., A. Nehammer, A. Middleton, M. Houseman, G. R. Taylor, M. Bitner-Glindzicz, G. Van Camp, M. Parker, I. D. Young, A. Davis, V. E. Newton, and N. J. Lench. 1999. Congenital non-syndromal sensorineural hearing impairment due to Connexin 26 gene mutations-molecular and audiological findings. *International Journal of Pediatric Otorhinolaryngology* **50**:3.
- Naarden, K. V., p. Decoufle, and K. Caldwell. 1999. Prevalence and characteristics of children with serious hearing impairment in Metropolitan Atlanta, 1991-1993. *Pediatrics* **103**:570-575.
- Naeem, Z., and V. Newton. 1996. Prevalence of sensorineural hearing loss in Asian children. *Br.J.Audiol.* **30**:332-339.

- Newton, V. E. 1985. Aetiology of bilateral sensorineural hearing loss in young children. *Journal of Laryngology and Otology*. Supplement **10**:1-57.
- Neyroud, N., F. Tesson, I. Denjoy, M. Leibouici, C. Donger, J. Barhanin, S. Fauré, F. Gary, P. Coumel, C. Petit, K. Schwartz, and P. Guicheney. 1997. A novel mutation in the potassium channel gene KvLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nature Genetics* **15** :186-189.
- NHS Executive. Piloting the introduction of universal neonatal hearing screening in England. 2004.
Ref Type: Unenacted Bill/Resolution
- Office for national statistics. Key Statistics, 2001 Census. <http://www.statistics.gov.uk>. 2001.
Ref Type: Report
- Office of National Statistics. Neighbourhood statistics: Tower Hamlets. 2001.
Ref Type: Report
- Parker, M. J., H. M. Fortnum, I. D. Young, and A. C. Davis. 2000. Genetics and deafness: what do families want? *J. Med. Genet.* **37**:26.
- Parving, A., and A. Hauch. 1994. The causes of profound hearing impairment in schools for the deaf-a longitudinal study. *Br. J. Audiol.* **28**:63-69.
- Parving, A., and V. Newton. 1995. Editorial: Guidelines for description on inherited hearing loss. *J. Audiol. Med* **4**:ii-v.
- Phelan, P., and T. A. Starich. 2001. Innexins get into the gap. *Bioessays* **23**:388-396.
- Powers, S. 1996. Deaf pupil's achievement in ordinary schools. *J. Brit. Assn. Teachers of the deaf* **20**:111-123.
- Preciado, D. A., L. H. Y. Lim, A. P. Cohen, C. Madden, D. Myer, C. Ngo, J. K. Bradshaw, L. Lawson, D. I. Choo, and J. H. Jr. Greinwald. 2004. A diagnostic paradigm for childhood idiopathic sensorineural hearing loss. *Otolaryngology - Head and Neck Surgery* **131**:804-809.
- Pressman, L., S. Pipp-Siegel, C. Yoshinaga-Itano, and A. Deas. 1999. Maternal sensitivity predicts language gain in preschool children who are deaf and hard of hearing. *J. Deaf Studies Deaf Education* **4**:304.
- Probst, F. J., R. A. Fridell, and Y. Raphael. 1998. Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene. *Science* **280**:1444-1447.
- Rabionet, R., P. Gasparini, and X. Estivill. 2000. Molecular genetics of hearing impairment due to mutations in gap junction genes encoding beta connexins. *Hum. Mutat.* **16**:190-202.

- Read, A. P. 1996. Basic genetic mechanisms. Pages 18-32 in A. Martini, A. Read, and D. Stephens editors. Genetics and hearing impairment. Whurr Publishers Ltd., London.
- Research & Scrutiny. Borough Profile updates: Bulletin 1; Demographics and Household trends. 1-16. 2004.
- Ref Type: Report
- Richard, G. 2003. Connexin gene pathology. Clin.Exp.Dermatol. **28**:397-409.
- Richard, G., T. W. White, L. E. Smith, R. A. Bailey, J. G. Compton, D. L. Paul, and S. J. Bale. 1998. Functional defects of Cx26 resulting from a heterozygous missense mutation in a family with dominant deaf mutism and palmoplantar keratoderma. Human Genetics **103**:393-399.
- Rickard, S., D. P. Kelsell, T. Sirimanna, K. Rajput, B. MacArdle, and M. Bitner-Glindzicz. 2001. Recurrent mutations in the deafness gene GJB2 (Connexin 26) in British Asian families. Journal of Medical Genetics **38**:530-533.
- Robinshaw, H. M. 1995. Early intervention for hearing impairment: Differences in the timing of communicative and linguistic development. British Journal of Audiology **29**:315-334.
- Rothrock, C. R., A. Murgia, E. L. Sartorato, E. Leonardi, S. Wei, S. L. Lebeis, L. E. Yu, J. L. Elfenbein, R. A. Fisher, and K. H. Fredrici. 2003. Connexin 26 35delG does not represent a mutational hotspot. Human Genetics **113**:18.
- Roux, A., N. Pallares-Ruiz, A. Vielle, V. Faugere, C. Templin, D. Leprevost, F. Artieres, G. Lina, N. Molinari, P. Blanchet, M. Mondain, and M. Claustres. 2004. Molecular epidemiology of DFNB1 deafness in France. BMC Medical Genetics **5**:1-10.
- Ryan, M., Z. Miedzybrodzka, L. Fraser, and M. Hall. 2003. Genetic information but not termination: pregnant women's attitudes and willingness to pay for carrier screening for deafness genes. Journal of Medical Genetics **40**:1-5.
- Saez, J. C., V. M. Berthoud, M. C. Branes, A. D. Martinez, and E. C. Beyer. 2003. Plasma membrane channels formed by connexins: their regulation and functions. Physiol.Rev. **83**:1359-3400.
- Sarno, C. N., and J. D. Clemis. 1980. A workable approach in the identification of neonatal hearing impairment. Laryngoscope **90**:1313-1319.
- Schrijver, I. 2004. Hereditary non-syndromic sensorineural hearing loss: Transforming silence to sound. Journal of Molecular Diagnostics **6**:275-284.
- Schulte, B. A., and J. C. Adams. 1989. Immunohistochemical localization of vimentin in the gerbil inner ear. J Histochem Cytochem **37**:1787-1797.

- Scott, D. A., M. L. Kraft, and R. Carmi. 1998. Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. *Human Mutation* **11**:387-394.
- Self, T., T. Sobe, N. G. Copeland, N. A. Jenkins, K. B. Avraham, and K. P. Steel. 1999. Role of myosin VI in the differentiation of cochlear hair cells. *Dev.Biol.* **214**:331-341.
- Simon, A. M., and D. A. Goodenough. 1998. Diverse functions of vertebrate gap junctions. *Trends Cell Biol.* **8**:477-483.
- Sinnathuray, A. R., J. G. Toner, A. Geddis, J. Clarke-Lytle, C. C. Patterson, and A. E. Hughes. 2004. Auditory perception and speech discrimination after cochlear implantation in patients with connexin 26 (GJB2) gene-related deafness. *Otology & Neurotology* **25**:930-934.
- Snoeckx, R. L., P. L. Huygen, D. Feldmann, S. Marlin, F. Denoyelle, J. Waligora, M. Mueller-Malesinska, A. Pollak, R. Ploski, A. Murgia, E. Orzan, P. Castorina, U. Ambrosetti, E. Nowakowska-Szyrwinska, J. Bal, W. Wiszniewski, A. R. Janecke, D. Nekahm-Heis, P. Seeman, O. Bendova, M. A. Kenna, A. Frangulov, H. L. Rehm, M. Tekin, A. Incesulu, H. H. Dahl, D. du Sart, L. Jenkins, D. Lucas, M. Bitner-Glindzicz, K. B. Avraham, Z. Brownstein, I. del Castillo, F. Moreno, N. Blin, M. Pfister, I. Sziklai, T. Toth, P. M. Kelley, E. S. Cohn, L. Van Maldergem, P. Hilbert, A. F. Roux, M. Mondain, L. H. Hoefsloot, C. W. Cremers, T. Lopponen, H. Lopponen, A. Parving, K. Gronskov, I. Schrijver, J. Roberson, F. Gualandi, A. Martini, G. Lina-Granade, N. Pallares-Ruiz, C. Correia, G. Fialho, K. Cryns, N. Hilgert, P. Van de Heyning, C. J. Nishimura, R. J. Smith, and G. Van Camp. 2005. GJB2 mutations and degree of hearing loss: a multicenter study. *American Journal of Human Genetics* **77**:945-957.
- Sobe, T., S. Vreugde, H. Shahin, M. Berlin, N. Davis, M. Kanann, Y. Yaron, A. Orr-Urtreger, M. Frydman, M. Shohat, and K. B. Avraham. 2000. The prevalence and expression of inherited connexin 26 mutations associated with nonsyndromic hearing loss in the Israeli population. *Human Genetics* **106**:50-57.
- Steel, K. P. 1998. A new era in genetics of deafness. *New England Journal of Medicine* **339**:1545.
- Steel, K. P., and C. J. Kros. 2001. A genetic approach to understanding auditory function. *Nature Genetics* **27**:143-149.
- Stephens, D. 2001. Audiological Terms. Pages 9-14 in A. Martini, M. Mazzoli, A. Read, and D. Stephens editors. *Definitions, Protocols and Guidelines in Genetic Hearing Impairment*. Whurr publishers, London.
- Stern, S. J., K. S. Arnos, L. Murrelle, K. Oelrich Welch, W. E. Nance, and A. Pandya. 2002. Attitudes of deaf and hard of hearing subjects towards genetic testing and prenatal diagnosis of hearing loss. *J.Med.Genet.* **39**:449-453.

- Stevens, J. C., D. M. Hall, A. Davis, C. M. Davies, and S. Dixon. 1998. The costs of early hearing screening in England and Wales. *Archives of Disease in Childhood* **78**:14-19.
- Stevens, J. C., and G. Parker. 2002. Screening and surveillance. Pages 146-168 *in* V. Newton editor. *Paediatric Audiological Medicine*. Whurr publishers, London.
- Stevens, J. C., H. D. Webb, J. Hutchinson, J. Connell, M. F. Smith, and J. T. Buffin. 1991. Evaluation of click-evoked otoacoustic emissions in the newborn. *British Journal of Audiology* **25**:11-14.
- Stevenson, V. A., M. Ito, and J. M. Milunsky. 2003. Connexin-30 deletion analysis in connexin 26 heterozygotes. *Genet.Test* **7**:154.
- Strachan, T., and A. P. Read. 1999. Molecular pathology. Pages 377-400 *in* T. Stracan, and A. P. Read editors. *Human molecular genetics*. Bios Scientific Publishers Ltd., Oxford.
- Sylhet Corp. The Sylhet City Corporation. 2005.
Ref Type: Report
- Tyson, J., L. Tranebjærg, S. Bellman, C. Wren, J. F. N. Taylor, J. Bathen, B. Aslaksen, S. J. Sørland, O. Lund, S. Malcolm, M. Pembrey, S. Bhattacharya, and M. Bitner-Glindzicz. 1997. IsK and KvLQT1: mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome. *Human Molecular Genetics* **6**:2179-2185.
- Uus, K., and A. C. Davis. 2000. Epidemiology of Permanent Childhood Hearing Impairment in Estonia, 1985-1990. *Audiology* **39**:192-197.
- Vallely, P. J., P. E. Klapper, and G. M. Cleator. 2002. Infectious causes of paediatric hearing impairment. Pages 183-210 *in* V. Newton editor. *Paediatric Audiological Medicine*. Whurr Publishers, London.
- Van Laer, L., P. Coucke, R. F. Mueller, G. Caethoven, K. Flothmann, S. D. Prasad, G. P. Chamberlin, M. Houseman, G. R. Taylor, C. M. Van de Heyning, E. Fransen, J. Rowland, R. A. Cucci, R. J. Smith, and G. Van Camp. 2001. A common founder for the 35delG GJB2 gene mutation in Connexin 26 hearing impairment. *J.Med.Genet.* **38**:515-518.
- Vanniasagaram, I., P. Tungland, and S. Bellman. 1993. A 5 year review of children with deafness in a multiethnic community. *J Audiol Med* **2**:9-19.
- Vetter, D. E., J. R. Mann, P. Wangemann, J. Liu, K. J. McLaughlin, F. Lesage, D. C. Marcus, M. Lazdunski, S. F. Heinmann, and J. Barhanin. 1996. Inner ear defects induced by null mutation of the *ISK* gene. *Neuron* **17**:1251-1264.

- Wei, C., X. Xu, and C. W. Lo. 2004. Connexins and cell signalling in developmental disease. *Annu.Rev.Cell Dev.Biol.* **20**:811-838.
- Wilcox, S. A., K. Saunders, A. H. Osborn, A. Arnold, J. Wunderlich, T. Kelly, V. Collins, L. J. Wilcox, R. J. McKinlay Gardner, M. Kamarinos, B. Cone-Wesson, R. Williamson, and H. H. Dahl. 2000. High frequency hearing loss correlations with mutations in the GJB2 gene. *Human Genetics* **106**:399-405.
- Willecke, K., J. Eiberger, J. Degen, D. Eckardt, and A. Romualdi. 2002. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol.Chem.* **383**:725-737.
- Yeager, M. 1998. Structure of cardiac gap junction intercellular channels. *J.Structural Biology* **121**:231-245.
- Ylikoski, J., U. Pirvola, and E. Lehtonen. 1992. Distribution of F-actin and fodrin in the hair cells of the guinea pig cochlea as revealed by confocal fluorescence microscopy. *Hear.Res.* **60**:80-88.
- Yoshinaga-Itano, C., D. Coulter, and V. Thomson. 2000. The Colorado Newborn Hearing Screening Project: Effects on speech and language development for children with hearing loss. *Journal of Perinatology* **20**:S132-S137.
- Yoshinaga-Itano, C., A. L. Sedey, D. K. Coulter, and A. L. Mehl. 1998. Language outcomes in early and later identified children with hearing loss. *Pediatrics* **102**:1161-1166.
- Zheng, J., W. Shen, D. Z. He, K. B. Long, L. D. Madison, and P. Dallos. 2000. Prestin is the motor protein of cochlear outer hair cells. *Nature* **405**:149-155.